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INVESTIGATIONS IN FISH CONTROL

DIVISION OF FISHES
U. S. NATIONAL MUSEUM

- 1. Laboratories and methods
for screening fish-control chemicals**
- 2. Preliminary observations
on the toxicity of antimycin A
to fish and other aquatic animals**



United States Department of the Interior
Fish and Wildlife Service
Bureau of Sport Fisheries and Wildlife

UNITED STATES DEPARTMENT OF THE INTERIOR

Stewart L. Udall, *Secretary*

Frank P. Briggs, *Assistant Secretary for Fish and Wildlife*

FISH AND WILDLIFE SERVICE

Clarence F. Pautzke, *Commissioner*

BUREAU OF SPORT FISHERIES AND WILDLIFE

Daniel H. Janzen, *Director*

The United States Department of the Interior, created in 1849, is concerned with management, conservation, and development of the Nation's water, wildlife, fish, mineral, forest, and park and recreational resources. It has major responsibilities also for Indian and Territorial affairs.

As America's principal conservation agency, the Department works to assure that nonrenewable resources are developed and used wisely, that park and recreational resources are conserved for the future, and that renewable resources make their full contribution to the progress, prosperity, and security of the United States, now and in the future.

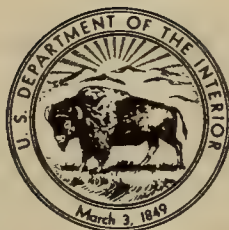
INVESTIGATIONS IN FISH CONTROL

1. Laboratories and methods for screening fish-control chemicals

Bureau Circular 185

2. Preliminary observations on the toxicity of antimycin A to fish and other aquatic animals

Bureau Circular 186



Washington, D.C. • June 1964

Investigations in Fish Control are reports on the results of work at the Bureau's Fish Control Laboratories at La Crosse, Wis., and Warm Springs, Ga. The two reports presented here are the first of several reports that are planned on work now under way.

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INVESTIGATIONS IN FISH CONTROL

1. Laboratories and methods for screening fish-control chemicals

By Robert E. Lennon, Fishery Research Biologist
Charles R. Walker, Chemist
Bureau of Sport Fisheries and Wildlife
La Crosse, Wis.



Bureau of Sport Fisheries and Wildlife

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Laboratories and methods for screening fish-control chemicals

By Robert E. Lennon, Fishery Research Biologist
and Charles R. Walker, Chemist
Bureau of Sport Fisheries and Wildlife
La Crosse, Wis.

Abstract.--This report describes the physical and technical facilities and the procedures of the Fish Control Laboratories at La Crosse, Wis., and Warm Springs, Ga. The laboratories emphasize screening of chemicals to find a variety of fishery management tools. Preliminary Screening ascertains whether a chemical in three concentrations has a desirable biological activity on eight species of fish in reconstituted water at 12° and 17° C. Delineative Screening ascertains effective concentrations (EC100) on eight species in reconstituted water (the method for which is described) at 12°, 17°, 22°, and 27°. Intensive Screening of promising fish-control agents ascertains effects on 24 species of fish and on other aquatic organisms, at different temperatures and in waters of various qualities, in the laboratory and in the field.

Fish Control Laboratories were established by the Bureau of Sport Fisheries and Wildlife at La Crosse, Wis., in 1959 and at Warm Springs, Ga., in 1963. The mission of the Laboratories is the development of means for efficient manipulation of fresh-water fish. In particular, safe and economical controls--chemical, biological, electrical, or mechanical--are sought for undesirable populations in standing and flowing waters. The objectives are sufficiently broad to encompass investigation and development of any new tools that may be useful in fishery management, fish culture, or fishery research.

At La Crosse, the buildings of the National Fish Hatchery were remodeled and expanded in 1960-62 to provide a large research facility (figs. 1 and 2). The subsidiary Laboratory at Warm Springs is new construction on the grounds of the National Fish Hatchery (fig. 3). Their locations offer contrasting advantages to be exploited through close coordination in the research on fish control:

La Crosse

Northern fishes.
Cold climate.
Cold water.
Hard water.

Warm Springs

Southern fishes.
Warm climate.
Warm water.
Soft water.

In equipping and staffing the laboratories, early recognition was given to the potentials of chemical control agents. The bioassay (wet), chemistry, and physiology laboratories are concerned with general and selective toxicants, attractants, repellants, anesthetics, sterilants, spawning inducers, osmoregulators, marking dyes, medications for diseases, and sedatives and decontaminants for fish distribution. Emphasis is on finding selective toxicants for longnose and shortnose gars, gizzard shad, goldfish, carp, squawfishes, white sucker, black bullhead, rock bass, green sunfish, pumpkinseed, yellow perch, and freshwater drum.

Ample justification for research on selective piscicides is contained in fishery literature.



Figure 1.--The Fish Control Laboratory at La Crosse, Wis.



Figure 2.--The fish holding house at La Crosse, Wis.



Figure 3.--The Fish Control Laboratory (foreground) and wet laboratory holding house at Warm Springs, Ga. Construction and grading were incomplete when the photograph was taken.

LesVeaux (1959) listed 30 States that need control of certain troublesome fishes. Among the qualities desired in selective toxicants are specificity to certain life stages or to certain fish, low cost, ease and safety of application, rapid degradation to nontoxic residues, harmlessness to warm-blooded animals, and effectiveness at low temperatures. Applegate et al. (1961) reported on an effective and selective sea lamprey larvicide. Loosanoff, MacKenzie, and Shearer (1960) showed the possibilities of controlling certain shellfish predators with chemicals in marine environments. These and other studies stimulated interest in investigations to find selective toxicants for various fresh-water fish. The American Fisheries Society, for example, resolved at its 88th annual meeting in 1958 to recommend, to the Secretary of the Interior, an expansion of research in fish control. Congress in the same year made the first appropriation for establishment of the Fish Control Laboratory at La Crosse.

FACILITIES

Bioassay laboratories

At La Crosse and Warm Springs there are wet laboratories for large-scale screening of

chemicals against fish. Fiberglass or aluminum troughs serve as water baths for bioassay vessels, and fiberglass or concrete tanks hold selected fish for experiments (figs. 4 and 5). Ground water is used for the water baths and fish holding, and temperatures are adjusted by means of thermostatically controlled immersion heaters or refrigeration units.

Deionized water of at least 1 million ohms resistivity is reconstituted according to a formula developed at the Bureau's Fish-Pesticide Research Laboratory and is employed as a test medium in the bioassay vessels. The following chemicals are added per liter of deionized water: 30 mg. of calcium sulfate, 30 mg. of magnesium sulfate, 48 mg. of sodium bicarbonate, and 3 mg. of potassium chloride.

Glass vessels are preferred for bioassays in the laboratory. Most of the screening is done in economical 1-gallon pickle jars which are used once and discarded. Five-gallon water buckets of glass are employed in advanced screening. They are reused after thorough decontamination and washing which include several steps:

- a. Rinse jar with tap water.

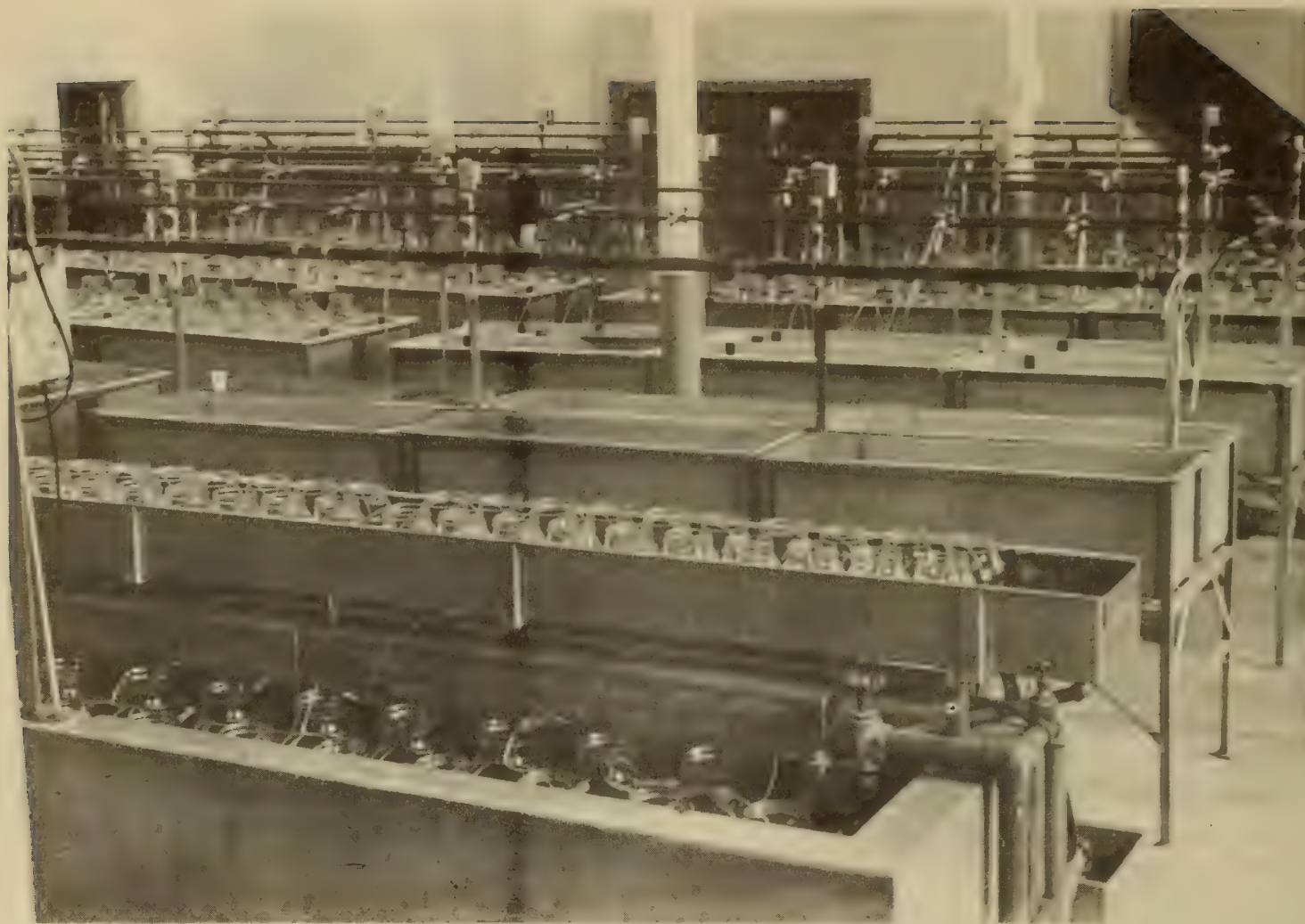


Figure 4.--View of wet laboratory at La Crosse showing batteries of bioassay vessels in concrete, aluminum, and fiberglass tanks.

- b. Add 6.3 grams of activated charcoal (1 gram/3 liters), fill jar with deionized water, and let stand over night.
- c. Empty and rinse; wash with strong detergent in hot tap water and rinse thoroughly.
- d. Sponge entire jar with 10- to 14-percent hydrochloric acid and rinse twice with deionized water.

Whenever residual contamination is detected or suspected, the jar is discarded. All discarded bioassay glassware is smashed to prevent further use.

All test solutions are discarded into a floor drain which continually carries at least 300 g.p.m. of waste water from the fish holding tanks. The dilution has been found to be more than sufficient to eliminate hazards.

All fish used in the bioassays are disposed of in gas-fired incinerators of complete-combustion type.

Other laboratory facilities

Each Fish Control Laboratory has chemistry, biochemistry, and physiology laboratories as adjuncts to the bioassay facilities. Chemicals for testing are received in the chemistry laboratories, stored in fire- and explosion-resistant vaults, and prepared in proper solutions and dilutions for bioassay. Compounds showing promise as fish-control agents are investigated in the biochemistry laboratories to evolve methods for application, effective and economical formulations, possibilities for potentiation, means for minimizing side effects and hazards, and techniques for detoxification. In the final stages of development, a control agent is studied in the



Figure 5.--View of a wet laboratory at Warm Springs showing a battery of bioassay vessels in a fiberglass tank.

physiology laboratories to define its mode of action on fishes and other organisms, any chronic effects, the fate of residues in live animals, and the risks, if any, to consumers of treated fish.

Fish-holding facilities

Large quantities of fish are required for the chemical screening programs. At La Crosse, for example, 498,000 fish of 34 species were used in 1963. Most are obtained from Federal, State, or private hatcheries and rearing stations; it is more satisfactory to arrange small, frequent deliveries than attempt to maintain large quantities on hand

for long periods in usable, disease-free condition. A holding house and outside pools are provided at each Laboratory for the maintenance, feeding, sorting, and grading of the experimental fish (fig. 3 and 6).

Outdoor bioassay pools

An intermediate step between laboratory testing and field trials of promising fish-control agents is essential to detect and evaluate some of the physical or chemical factors that influence the performance of a candidate agent in natural waters. Raceways and portable plastic pools are located at each Laboratory for this purpose.



Figure 6.--Interior view of fish holding house at La Crosse.

Inexpensive vinyl wading pools, 9 and 10 feet in diameter, 2.5 feet deep, and about 1,000 gallons in capacity, are set up as described by Lawrence and Blackburn (1962) in outdoor testing areas (figs. 7 and 8). Bottom soils of various types, pond or ground waters, aquatic plants, invertebrates, fish, and amphibians are used in them as needed during chemical trials. Contaminated vinyl liners are economically replaced. The pools at La Crosse are employed only during the warm season, but those at Warm Springs are in operation all year.

Ten- and 20-foot concrete raceways are used for extraordinary tests in running or standing water. Disposable vinyl liners are used when necessary to avoid harmful contamination of the raceways.

METHODS

The static bioassay is the first approach in screening chemicals for control agents. Constant-flow bioassays are reserved for advanced stages of testing.

Bliss (1957) defined a bioassay as a determination of the potency of a physical, chemical, or biological agent by means of a biological indicator. Noting its development during the past 20 or 30 years by scientists from many and diverse fields, he listed principles which characterize the modern bioassay: (1) Potency is a property of the drug, not of the response; (2) potency is relative, not absolute; (3) the assayed potency of an unknown is only an estimate of its true value; and (4) both the reliability



Figure 7.--Vinyl bioassay pools on levee at La Crosse.



Figure 8.--Vinyl bioassay pools at Warm Springs.

and efficiency of an assay are linked inseparably with its design. Observance of these principles overcomes some major disadvantages of the bioassay as a research tool.

Fish have long been employed as biological indicators in bioassays of water pollutants, insecticides, herbicides, detergents, and other substances. The standards recommended by Doudoroff et al. (1951), Henderson and Tarzwell (1957), and Henderson (1960) for such tests have been widely accepted and applied, although difficulties in comparing studies have arisen because of the many kinds of fish involved. Douglas and Irwin (1962) pointed out that the results of independent bioassays often cannot be related because the comparative resistance of the many test fishes has not been established. They noted that certain species have been more useful in toxicity bioassays than others, and they emphasized the need for knowledge about the reactions of different species of fish when exposed to a particular toxicant.

The large body of literature on methods and results of bioassays with fish has been helpful in defining the screening programs of the Fish Control Laboratories. In general, the practical methods proposed by the investigators cited above are followed but with some modifications since we seek more in biological activities than acute toxicity only.

Test chemicals

The test chemicals are selected by staff chemists and biologists and are contributed by industry. Preference is given to compounds that have demonstrated biological activity or are suspected of possessing a useful activity against fish. It is also desirable to have as much information as possible before screening on the nature and properties of each chemical, its shelf life and stability, its solubility, and its potential hazards to investigators. Security is respected, and precautions are taken with compounds and test data to protect the rights of contributors.

The chemicals obtained for screening are arbitrarily classified as follows in order to

facilitate scheduling of tests, observation of responses, and reporting of results:

1. Natural organic products:
 - a. Animal extracts (steroids, proteins, etc.).
 - b. Plant extracts (rotenoids, alkaloids, phenols, etc.).
 - c. Fermentation products (antibiotics, etc.).
2. Synthetic organic products:
 - a. Halogenated hydrocarbons.
 - b. Nitrogen-bearing hydrocarbons (salicylanilides, carbamates, triazines, etc.).
 - c. Phosphorus-bearing hydrocarbons.
 - d. Sulfur-bearing hydrocarbons (mercapto, thioates, thiozines, etc.).
 - e. Miscellaneous compounds and combinations.
3. Inorganic products.

The progress of a chemical through testing and development is depicted in figure 9. A compound is introduced into Preliminary Screening to detect whether it has activity against fish. If it has, it is advanced into Delineative Screening, where the effective concentrations are defined and the possible usefulness of the substance is declared. If results are favorable, the candidate is referred to Intensive Screening, where it is fully evaluated in the laboratory and in the field as a fishery tool. Compounds which fail to meet requirements at any stage of screening are shelved or discarded immediately.

Test fishes

It is of utmost importance that the validity and comparability of the bioassays be assured by using only fish of selected species, of certain sizes, and in good condition as biological indicators. The criteria are defined and strictly observed for each stage of screening. Therefore, the rate of progress of a chemical through screening and development depends largely on the availability of the prescribed fishes.

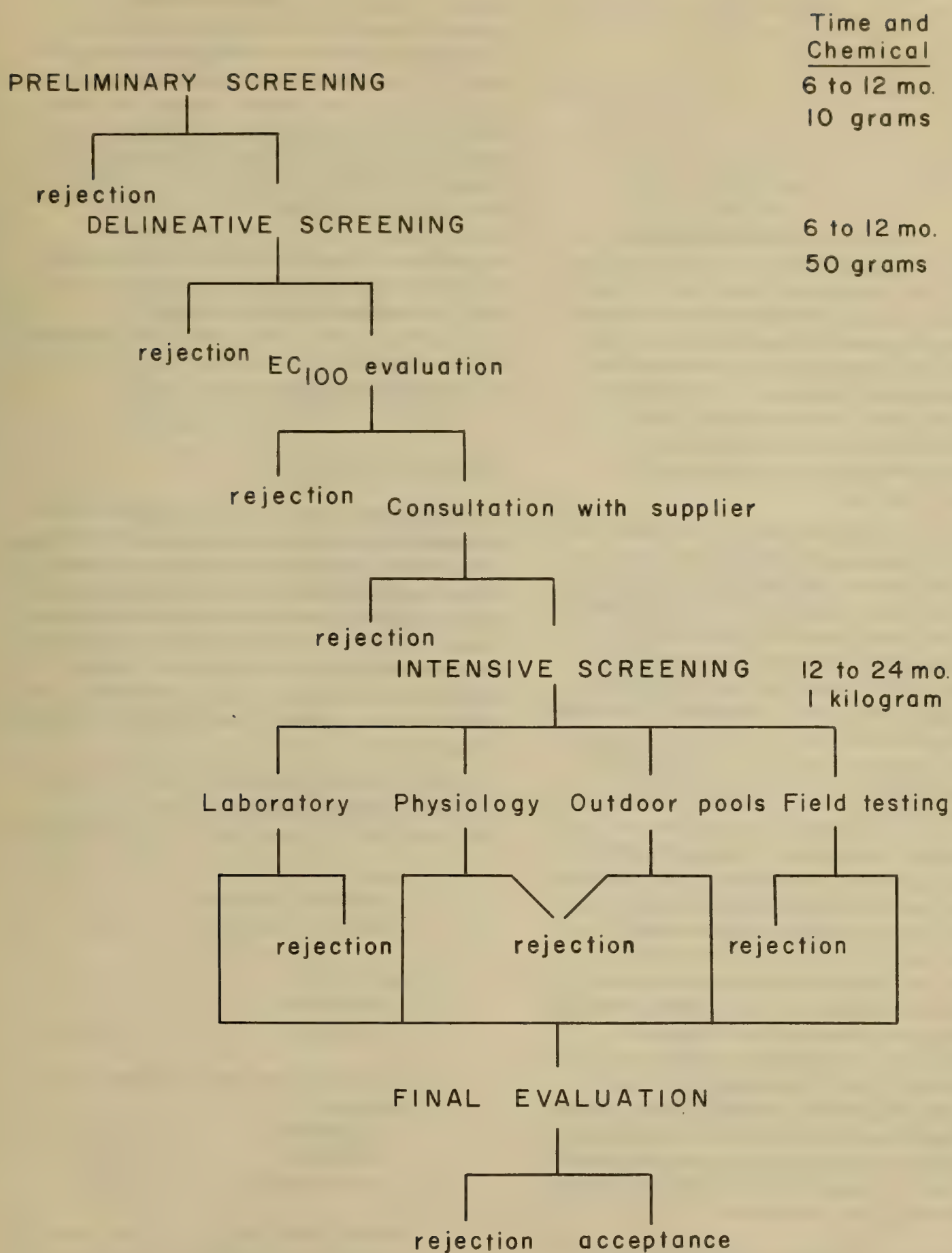


Figure 9.-Schematic on the progress of chemicals through screening.

TABLE 1.--Time required by certain fish held at 12° C. to empty the digestive tracts after food is withheld

Species	Number of fish		Voiding time (hours)
	Tested	Per pound	
Rainbow trout.....	90	2,000	36
".....	30	380	60
".....	12	22	84
Goldfish.....	30	207	48
River shiner.....	30	226	36
White sucker.....	12	76	60
Green sunfish.....	45	355	60
Pumpkinseed.....	45	177	84
Bluegill.....	60	368	84
Longear sunfish.....	45	465	72

Lots of hatchery fish are requested for delivery at least 2 weeks prior to use in bioassays. They are placed in the care of fish culturists, and everything possible is done to minimize stresses. During the first 10 days they are fed, prophylactically or therapeutically treated as necessary, and observed to evaluate them as test animals. Lots in which the mortalities exceed 10 percent within the period are not moved into the bioassay program.

The fish for experiments are carefully graded to desired and uniform size and transferred into the wet laboratories or outside pools 3 or 4 days before use. Food is withheld for as long as 96 hours before screening, depending on the life stage and species of fish. Generally, young fish and certain species require less time to empty the intestinal tract than others (table 1). The fish spend at least 24 hours in water similar to the test medium before being introduced into the bioassay vessels.

A help in maintaining comparable results in bioassays is use of a recognized reference toxicant against a sample of fish from each test lot. We employ para-, para'-DDT (1,1,1-trichloro-2,2-bis(p-chlorophenyl) ethane), and the test is made coincidentally with the introduction of a lot of fish into the screening program; the test is repeated biweekly if the lot remains on hand. The sensitivity (EC50) of the sample is determined for comparison with regression curves established for the species from experience or literature. Although costly, the test provides the only measure of relative sensitivity of fish used in the bioassays.

The value of reference tests was demonstrated during shakedown trials of facilities

and methods at La Crosse. For example, goldfish from Missouri and Wisconsin had such widely divergent sensitivities that they bracketed those of other species. Also, the sensitivities within a lot of fish differ significantly if some specimens are exposed to presumably harmless electric shocks like those experienced in electrofishing. Moreover, the stress of disease, malnutrition, temperature change, or altered water quality may influence sensitivity to a toxicant. Holding time is another factor, and we observed that the sensitivities changed greatly in a lot of goldfish which was retained for 2 months. Saila (1953) emphasized the significance of this and showed that the resistance of his mosquito-fish to rotenone decreased in rough proportion to the length of time they were held.

Responses of test fish

We recognize that the observer may be the greatest source of error in bioassays with fish. The criteria of response are arbitrary and subject to individual interpretation. Moreover, they are complicated at the Fish Control Laboratories because effects in addition to and more subtle than acute toxicity are sought. To achieve individual and mutual consistency, the observers are trained to use definite criteria of response.

Chemicals may have either short-term or long-term effects on fishes. Those of short term are typically identified with the following:

1. Acute toxicity:
 - a. Selective toxicant.
 - b. General toxicant.
2. Movement:
 - a. Attractant.
 - b. Repellant.
3. Facilitating capture, handling, or transport:
 - a. Anesthetic.
 - b. Sedative.
 - c. Osmoregulator.
4. Marking live fish:
 - a. Immersion stain.
 - b. Internal stain.

5. Therapy or prevention of disease:

- a. Bactericide.
- b. Parasiticide.
- c. Fungicide.
- d. Prophylactic.
- e. Antiseptic.

The long-term activities of potential control chemicals may be difficult to detect and evaluate. They are associated with--

1. Control of reproduction:

- a. Hormonal spawning inducer.
- b. Hormonal spawning inhibitor.
- c. Sterilant.

2. Control of growth and development:

- a. Growth stimulant.
- b. Growth inhibitor.

The criteria employed to evaluate the responses of fish are rather refined, and are applicable to short-term responses. They are used as necessary at each observation period to identify fully the reactions of the fish. They are--

General behaviour:

- 1. No observable difference from control.
- 2. Quiescent.
- 3. Excitable.
- 4. Irritated.
- 5. Surfacing.
- 6. Sounding.
- 7. Twitching.
- 8. Motionless:
 - a. Tetany.
 - b. Flaccidity.
- 9. Swimming:
 - a. Erratic; convulsive.
 - b. Gyrating; skittering.

c. Inverted.

d. On side.

e. Against tank sides on bottom.

Integument:

1. Pigmentation:

- a. No observable change.
- b. Light discoloration.
- c. Dark discoloration.
- d. Varidiscoloration.

2. External mucosa:

- a. No observable change.
- b. Shedding; patchy.
- c. Copious exudate.
- d. Coagulation.

3. Hemorrhagic.

Respiration:

1. Respiratory rate:

- a. No observable change.
- b. Rapid.
- c. Slow.
- d. Irregular.
- e. Ceased.

2. Gulping air.

3. Structures, organs:

- a. No observable change.
- b. Mouth gaping.
- c. Hemorrhage in gills.
- d. Irritative response.
- e. Copious mucus in gills.

Alimentary responses:

1. No observable change.

2. Egurgitating mucus or other material.

3. Defecating mucus or other material.

Nervous responses:

1. No observable change.

2. Sensitivity to stimuli:

	<u>Positive</u>	<u>Negative</u>
a. Exterior movement..	_____	_____
b. Light.....	_____	_____
c. Sound.....	_____	_____
d. Touch.....	_____	_____
e. Electric probe	_____	_____

Moribundity:

1. No motion.
2. No respiration.
3. Distended operculum.
4. Opaque eyes.
5. Death.

Recovery:

1. Complete.
2. Incomplete.

Additional and more definitive observations are made of fish at the in-test, recovery, or postmortem stages in the biochemistry and physiology laboratories. Their objectives may be, for example, the modes of action and side effects of control agents.

Screening

Preliminary Screening.--Preliminary Screening is designed to detect whether a selected chemical at 0.1, 1, and 10 p.p.m. has a biological activity (see list of criteria above) against eight species of fish within 48 hours. It is a static bioassay in 1-gallon jars containing 2.5 liters of pre-aerated, reconstituted water. No artificial aerating is done during the test. The temperatures of water

baths are 12° C. at La Crosse and 17° C. at Warm Springs.

The fish include the following species:

<u>La Crosse</u>	<u>Warm Springs</u>
Rainbow trout.	Carp.
Goldfish.	Black bullhead.
White sucker.	Green sunfish.
Yellow perch.	Bluegill.

Goldfish from the same gene pool and from the same source are employed for occasional toxicity checks between the two laboratories.

At least 10 fish of each species are used with each concentration of chemical and as controls. They weigh between half a gram and 2 grams each, but the weight range of the fish in each test does not exceed 15 percent. They are distributed at one species per jar about 16 hours before the bioassay at a loading of 1 gram or less of fish per liter of test medium. Thus, there may be but one or two fish per jar, and up to 10 jars may be needed for each concentration of chemical.

A stock solution of a test compound is prepared within an hour of the bioassay. The solvents preferred are water, acetone, and ethanol, in that order. Because of their toxicity to fish, precautions are taken that the concentrations of acetone and ethanol do not exceed 4 parts per thousand in the bioassay media. Furthermore, whenever these solvents are used, equal volumes of them are applied in control vessels. Thus the volume in controls is equal to the highest volume used in a dosage series. Small aliquots of a stock solution are thoroughly mixed into bioassay media to avoid improper dilution or stratification.

The responses of the fish in bioassays and controls are observed routinely at 0.75, 1.5, 3, 6, 24, and 48 hours, but so far as possible they are recorded on the first day and thereafter as often as the nature of a candidate compound warrants.

If a compound has a desirable activity against the fish, it is held for further screenings, which are discussed briefly below.

Delineative Screening.--The effective concentrations (EC100) of a chemical against fish are determined in Delineative Screening. The vessels may be jars or troughs for static or flowing water trials. The water, aeration, species and size of fish, duration of tests, and controls are the same as in Preliminary Screening. A compound is first bioassayed at 12° C. to define concentrations which evoke all-or-none responses from the fish. The approach to these concentrations may be direct by bracketing and interpolation, or it may be indirect by probit analysis, whichever is the shorter. The EC100, if seemingly practical, is confirmed by seven replicate trials.

A chemical which yields favorable results at 12° C. is retested at 17°, 22°, and 27° to evaluate the effects of temperature on the effective concentrations. At 22° and 27°, rainbow trout are omitted from the bioassays, and the loading rates for other species are half a gram or less per liter of test medium.

If a compound succeeds in these trials, its potential as a fish-control agent is estimated. Information is sought on its possible application; on the source and manufacturing costs; on possible hazards, conflicts, or limitations in use; and on the size of the market. Only if it continues to appear promising is the chemical promoted into the more elaborate and expensive Intensive Screening.

Intensive Screening.--Intensive Screening is directed toward development of a promising chemical as a fish-control agent and the definition of its advantages and limitations. These are broad objectives, and some subdivision of approaches is in order.

A. Stages: The advanced tests of a compound are accomplished in the wet laboratories, in outside pools, and finally in the field. They may include static or flowing water bioassays with durations of 24, 48, and 96 hours or longer. The vessels may include 1-gallon jars with 2.5 liters of test medium, 5-gallon jars with 15 liters of medium, and fiberglass troughs of 4-, 8-, and 16-foot lengths. The outdoor pools include 1,000-gallon vinyl units and 10- and 20-foot concrete

raceways. Proportioning pumps are used in the flowing bioassays to achieve consistent concentrations of chemical.

The initial trials of a compound in the field are conducted in waters closed to public use. These waters are found, for example, on the grounds of Federal and State fish hatcheries, on wildlife refuges, or on military reservations. Trials are manned or immediately supervised by the Laboratory staff. Subsequent experiments in the field may be accomplished by selected cooperators in State and Federal agencies.

B. Varieties of fish: Sixteen species in addition to those included in Preliminary and Delineative Screening are employed. They are:

1. Brook trout.
2. Northern pike.
3. Fathead minnow.
4. Brook stickleback.
5. Pumpkinseed.
6. Longear sunfish.
7. Smallmouth bass.
8. Walleye.
9. Gizzard shad.
10. Golden shiner.
11. Bigmouth buffalo.
12. Brown bullhead.
13. Channel catfish.
14. Redear sunfish.
15. Largemouth bass.
16. White crappie.

Other species may be used on occasion in advanced tests.

C. Life stages: Various life stages of fish, from egg to mature adult, may be involved in the more advanced bioassays to detect the presence, absence, or variation of response to a promising chemical. It may be a great advantage or disadvantage if a potential control agent is selective for one life stage and not for another.

D. Other organisms: The effects of a potential chemical tool on other aquatic life must be assessed at least minimally in the laboratory before it is tested in the field. Some forms such as water fleas, snails, and plants

are cultured for this purpose. Others, such as freshwater scuds, damselfly and mayfly nymphs, and tadpoles, are collected in the field. Static bioassays in glass jars are conducted with chemical concentrations and experimental conditions similar to those of the fish assays.

E. Temperature: The influences of water temperature on the biological activity of a candidate chemical are defined for all species and different life stages from bioassays under ice and at 20°, 70°, 120°, 170°, 220°, and 270° C. It is especially desirable to develop compounds that may be applied effectively during cold seasons when recreational use of public waters is low.

F. Water quality: It is recognized that water quality may exert tremendous influence on the activity of an introduced chemical. Accordingly, the effectiveness of a candidate control is observed in hard and soft waters, in alkaline and acid waters, and in waters of low and high organic content, in the laboratory and field. Initially, the formula for reconstituting deionized water is altered to detect trends.

G. Formulations: Formulation is often the key to an effective biological activity or efficient application of a compound. Density and solubility, for example, may be extremely important factors in a fish-control agent. Various formulations with carriers, wetting agents, dispersing agents, potentiators, or inhibitors may be prepared or acquired for testing. Combinations of biologically active chemicals are tested to ascertain their potentials as multiple controls for fish and aquatic weeds, fish and parasites, or fish and amphibians.

Moreover, the practicality of a control agent might depend on or be enhanced by an applicator's ability to modify or arrest its activity efficiently at a given point. Hence, the Intensive Screening includes experiments with possible deactivators, detoxifiers, or decontaminants for potential controls.

Unsatisfactory results or hazardous side effects, and new information on manufacturing, marketing, or competitive control

chemicals may contribute at any point in Intensive Screening to the abandonment of further trials and development.

Recording and reporting results

The results obtained with each chemical are analyzed soon after completion of the Preliminary, Delineative, and Intensive Screenings. They are furnished to the contributor of the chemical immediately. After screening, we evaluate the compound as a potential fishery management tool, to determine patent positions, to refer it for studies of residues and chronic effects, and to investigate requirements for clearance and labeling.

For compounds with negative results the screening data are segregated according to the classes of chemicals involved and will be published as soon as the accumulation of data warrants. The results with chemicals which succeed as fish-control agents will be reported individually and addressed primarily to fish managers or fish culturists. Thus, we plan to define the range of effective concentrations for certain target fishes in waters of different temperatures, different types, and of various qualities.

SUMMARY

The Fish Control Laboratories at La Crosse, Wis., and Warm Springs, Ga., are equipped to find and develop chemicals which may be used as fishery management tools. Objectives of the research are general and selective toxicants, attractants, repellents, anesthetics, sterilants, spawning inducers, marking dyes, medications for diseases, and sedatives for fish distribution. The facilities include chemistry, physiology, and wet laboratories; fish holding structures; and outdoor pools and raceways. The program involves three stages of chemical screening.

Preliminary Screening detects whether a selected chemical at 0.1, 1, and 10 p.p.m. has an activity in static bioassays on eight species of fish in reconstituted water at 120° and 170° C.

Delineative Screening defines the effective concentrations (EC100) of a chemical in static or flowing bioassays on eight species of fish in reconstituted water at 12°, 17°, 22°, and 27° C.

Intensive Screening is reserved for chemicals which show great promise as fish-control agents. Effective concentrations are determined in the laboratory, in outdoor pools, and in the field against 24 or more species of fish in various life stages from egg to adult, against selected aquatic organisms, and in waters of different temperatures and various qualities. The modes of action, side effects, chronic effects, and deactivators also are studied, as necessary.

The results of screening are reported direct to contributors. They are also published by the Bureau. The effective concentrations of fishery tools on target fishes under certain conditions are given.

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INVESTIGATIONS IN FISH CONTROL

2. Preliminary observations on the toxicity of antimycin A to fish and other aquatic animals

By Charles R. Walker, Chemist
Robert E. Lennon, Fishery Research Biologist
Bernard L. Berger, Chemist
Bureau of Sport Fisheries and Wildlife



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Preliminary observations on the toxicity of antimycin A to fish and other aquatic animals

By Charles R. Walker, Chemist
Robert E. Lennon, Fishery Research Biologist
and Bernard L. Berger, Chemist
Bureau of Sport Fisheries and Wildlife

Abstract.--Antimycin A, an antifungal antibiotic, has been suggested for use as a fish toxicant. Preliminary tests were made to evaluate its effects at concentrations of 0.01 to 120 p.p.b. on 24 species of freshwater fish in the laboratory and 25 species in outdoor pools. Responses of a select group of other animals and aquatic plants are discussed. The antibiotic is a powerful fish toxicant. Carp and other rough fish were killed by small concentrations in short exposures at cool and warm temperatures. Longnose gar, bowfin, black bullheads, and yellow bullheads were relatively resistant to the quantities tested. Plankton, aquatic plants, bottom fauna, salamanders, tadpoles, and turtles were not harmed by piscicidal concentrations. Antimycin A degrades rapidly in water, especially in the presence of free hydroxide. Detoxification occurred within 24 to 96 hours. Further studies are planned on the performance of antimycin A against various life stages of fish, on other aquatic animals, and in waters of differing qualities and temperatures. The process of detoxification and the fate of residues deserve further attention.

An objective of the Fish Control Laboratories is the development of new fish toxicants that can be used safely and economically in the management of fish populations. Antimycin A exhibits properties desired in a candidate fish toxicant. It is lethal to certain target fishes in low concentration and on short exposure; it works in cool and warm water and in the presence of aquatic plants; it degrades rapidly in water and appears to leave no harmful residue.

This report summarizes data obtained on antimycin A in the laboratory and small outdoor pools and larger hatchery ponds. Development and efficacy of the compound as a fishery tool is to be further investigated.

ANTIMYCIN

Sources and uses

Antimycin is an antifungal antibiotic isolated from the bacteria *Streptomyces* sp. and identified by Dunshee, Leben, Keitt, and Strong (1949) at the University of Wisconsin. Following this discovery, at least seven species of *Streptomyces* were found to be producers of antimycin. Burger, Teitel, and Grunberg crystalized the antibiotic from two species of *Streptomyces* (Strong, 1956). Later at the University of Wisconsin, another culture produced an antimycin-like product which showed promise as an antibiotic for plant pathogens (Lockwood et al., 1954).

Harada and associates (Nakayama et al., 1956) in Japan discovered an antimycin-producing culture of *Streptomyces kitazawaensis* which differed from the first culture at the University of Wisconsin, but both produce an antitumor substance (*carzinomyceticus*). Research at the University of Tokyo by Watanebe et al. (1957) on *S. blastmyceticus* yielded an antibiotic called blastmycin which consists largely of antimycin A₃. Harada et al. (1959) devoted special attention to the antifungal property of blastmycin as a control for rice blast disease (*Piricularia oryzae*) in Japan.

Derse and Strong (1963) related that antimycin is an antibiotic of unusual chemical structure which is toxic to yeasts, other fungi, insects, and mammals, but not to bacteria. They also reported that it is extremely toxic to goldfish at 1 p.p.b. On the basis of this observation, on the rapid degradation of the chemical, and its much lower toxicity to higher animals, they suggested that antimycin may be useful in fish management.

Composition and structure

The complex structure of antimycin was elucidated by Dunshee et al. (1949), Tener et al. (1953), Strong (1956) and Strong et al. (1960), van Tamelen et al. (1959 and 1961), and Dickie et al. (1963). It is illustrated in figure 1.

Lockwood et al. (1954) described antimycin as a complex made up of several active fractions which they identified from paper chromatograms as A₁, A₂, A₃, and A₄ according to increasing R_F values. Liu and Strong (1959) determined that one or more of these R_F values were represented in antimycin A-35, antimycin A-102, blastmycin, and virosin, and they investigated them. Further study by Dickie and his associates (1963) established that the fractions differ only in the alkyl side chain (R) in figure 1. The antimycin A₁ and A₄ fractions are probably isomeric with R = n-hexyl, and calculations of the elemental composition indicate that the empirical formula is C₂₈H₄₀N₂O₉. The A₂ and A₃ isomers bear the n-butyl side chain, and the empirical formula is perhaps C₂₆H₃₆N₂O₉. The percentage composition

of fractions or isomers is very important to the biological activity of the antimycin complex.

Physical and chemical properties

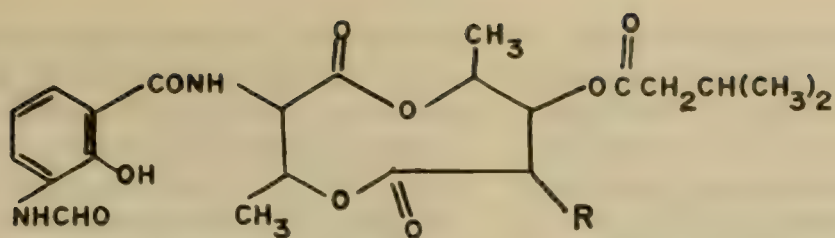
The fermentation extracts of antimycin are dark, tarry substances which upon further purification yield a fine crystalline material. This nitrogenous, phenolic complex is characterized by solubility in polar organic solvents including ethanol, acetone, and chloroform; slight solubility in nonpolar solvents including petroleum ether, benzene, and carbon tetrachloride; and relative insolubility in water and 5-percent solutions of hydrochloric acid, sodium bicarbonate, and sodium carbonate (Keitt, Leben, and Strong, 1953).

The infrared absorption spectrum of antimycin has been identified in isolates from several cultures, although the crystalline products appear to have different properties. These differences are attributed to the intricate composition of the antibiotic and the presence of impurities associated with samples (Strong, 1956). For example, blastmycin has almost the duplicate IR spectrum of antimycin A-35 isolate, but the melting points are 166°-167° and 140.5°-141.5° C. respectively. Blastmycin is composed primarily of the antimycin A₃ fraction with a trace of A₄ in contrast to antimycin A-35, antimycin A-102, and virosin, which contain additional subcompounds A₁ and A₂ (Strong, 1956; Liu and Strong, 1959).

Antimycin is susceptible to alkaline degradation as indicated in figure 1. Hydrolytic cleavage occurs at the lactone carbonyl sites on the cyclic diester and leads to the formation of antimycic acid or blastmycic and the neutral fragment (van Tamelen et al., 1961; Liu et al., 1960; and Tener et al., 1953). The degradation is rapid in water, and detoxification of 10 p.p.b. is accomplished within 7 days according to Derse and Strong (1963); it is accelerated in the presence of light, high alkalinity, and warm temperatures.

Biological activity

Antimycin is a powerful and highly selective inhibitor of the electron transport in oxidative



Antimycin A₁ ; R = n-hexyl : C₂₈H₄₀N₂O₉

Antimycin A₃ ; R = n-butyl : C₂₆H₃₆N₂O₉

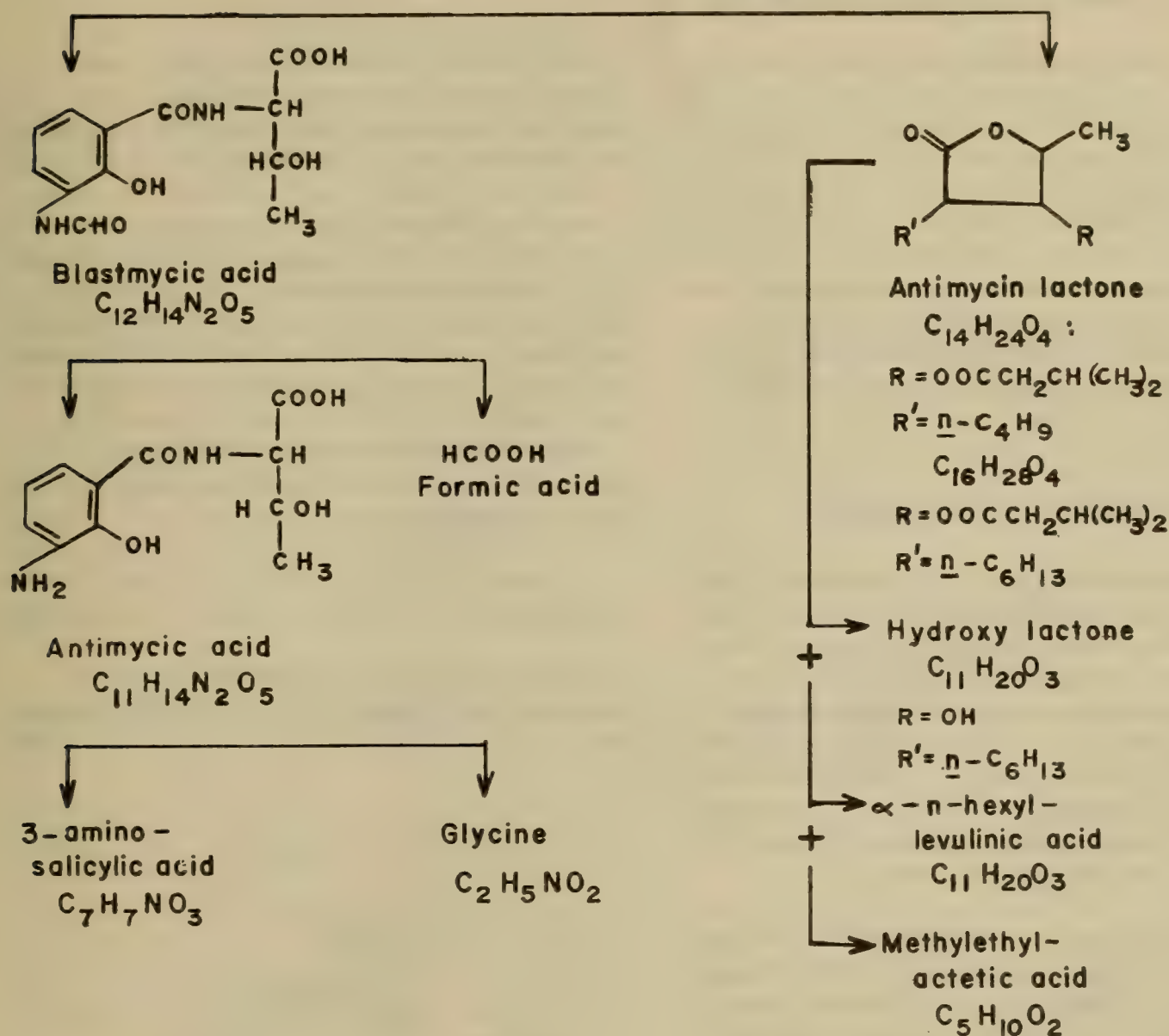
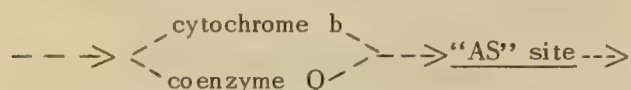


Figure 1.--Structure of antimycin and the assumed process of breakdown under alkaline conditions in the laboratory.

phosphorylation systems (Strong, 1956). It retards the respiration of cells, and the selective action in the electron transport chain at the cytochrome b - (Coenzyme Q) - cytochrome c has made antimycin an indispensable reagent for enzyme studies. Its effects on the succinic-oxidase system have been described as the "antimycin-A-blocked factor." Gottlieb and Ramachandran (1961) illustrated the site of action of antimycin and ascocin as follows:

Substrate--> Pyridine nucleotide--> Flavoprotein-->



cytochrome c--> cytochrome a--> oxygen

Because of its extreme potency as an inhibitor of electron transport, Derse and Strong (1963) surmised that antimycin is absorbed into the gills and interferes with respiration in fishes.

METHODS AND MATERIALS

Crystalline antimycin A was supplied by the Wisconsin Alumni Research Foundation from Kyowa Fermentation Company, Ltd., in Tokyo, Japan. This material was isolated from the culture of *Streptomyces kitazawaensis* and had the following fractions by weight: A₁, 40 percent; A₂, 20 percent; A₃, 20 percent; and A₄, 10 percent. Although the fraction A₃ amounts to only 20 percent, it accounts for about 60 percent of the biological activity.

Stock solutions were prepared with 100 milligrams of crystalline antimycin A dissolved in 1 liter of acetone. They were renewed with each series of bioassays, although tests indicated that solutions in acetone are relatively stable up to 24 days. Crystalline material stored at room temperature for 2 years also remained stable.

Laboratory tests

The methods and facilities employed for evaluation of potential fish-control agents were described by Lennon and Walker (1964). The

bioassays of antimycin A were conducted in slightly alkaline and medium hard, reconstituted water at 12°, 17°, and 22° C. Twenty-four species of fish, representing nine families, were included (table 1). They were supplied by national fish hatcheries, the Wisconsin Conservation Department, and Ozark Fisheries, Inc., and each lot was graded to a desired size before use.

Aliquots of the stock solution of antimycin A were diluted and stirred into the 1- or 5-gallon bioassay vessels in the presence of fish. The responses of the fish to the toxicant were observed at 24, 48, 72, and 96 hours.

Other animals included in bioassays were water fleas (*Daphnia magna*), crayfish (*Cambarus* sp.), damselfly nymphs (*Ischnura* sp.), tiger salamander (*Ambystoma tigrinum*), and bullfrog tadpoles (*Rana catesbiana*). They were stocked in bioassay vessels as follow: 10 water fleas or 2 damselfly nymphs in each 16-ounce jar, 1 crayfish or 2 bullfrog tadpoles in each 1-gallon jar, and 1 adult tiger salamander in each 5-gallon jar.

Field tests

Vinyl wading pools.--Only a few outdoor bioassays were made in 1962 and 1963 because only small quantities of toxicant were available.

TABLE 1.--The 24 fishes used in laboratory tests of antimycin A

Common name	Technical name	Size range (grams)
Gizzard shad.....	<i>Dorosoma cepedianum</i>	12.0-15.0
Rainbow trout.....	<i>Salmo gairdneri</i>	1.0- 1.6
Brown trout.....	<i>Salmo trutta</i>	1.2- 1.4
Northern pike.....	<i>Esox lucius</i>	0.5- 0.6
Stoneroller.....	<i>Camptostoma anomalum</i>	3.0- 4.0
Goldfish.....	<i>Carassius auratus</i>	1.5- 2.4
Carp.....	<i>Cyprinus carpio</i>	0.6- 2.3
Golden shiner.....	<i>Notemigonus crysoleucas</i>	1.0- 2.2
Fathead minnow.....	<i>Pimephales promelas</i>	0.9- 1.8
White sucker.....	<i>Catostomus commersoni</i>	1.3- 2.8
Bigmouth buffalo.....	<i>Ictiobus cyprinellus</i>	1.6- 2.5
Black bullhead.....	<i>Ictalurus melas</i>	0.7- 2.3
Yellow bullhead.....	<i>Ictalurus natalis</i>	1.2- 2.2
Channel catfish.....	<i>Ictalurus punctatus</i>	1.5- 1.8
Brook stickleback.....	<i>Eucalia inconstans</i>	0.6- 1.0
Green sunfish.....	<i>Lepomis cyanellus</i>	0.8- 2.5
Pumpkinseed.....	<i>Lepomis gibbosus</i>	1.0- 2.3
Bluegill.....	<i>Lepomis macrochirus</i>	1.2- 2.4
Longear sunfish.....	<i>Lepomis megalotis</i>	1.0- 2.5
Largemouth bass.....	<i>Micropterus salmoides</i>	1.8- 2.9
White crappie.....	<i>Pomoxis annularis</i>	1.5- 3.0
Iowa darter.....	<i>Etheostoma exile</i>	0.6- 1.2
Yellow perch.....	<i>Perca flavescens</i>	0.6- 3.0
Walleye.....	<i>Stizostedion vitreum</i>	0.4- 0.8

The test vessels were 1,000-gallon wading pools similar to those described by Lawrence and Blackburn (1962). Some physical, chemical, and biological conditions characteristic of ponds were simulated or intrinsic. The physical aspects included bottom soils of sand and loam, naturally varying temperatures, turbidity, and natural light. The chemistry of the well water in the pools was modified by physical and biological factors.

Of the 18 pools, 9 had 3 inches of sand on the bottom, and 9 had 3 inches of silt loam. After the pools were filled, the following were introduced: *Sagittaria latifolia*, *Elodea canadensis*, *Myriophyllum heterophyllum*, *Potamogeton nodosus*, *P. pectinatus*, *Spirogyra* spp., and phytoplankton. They were established, and the water chemistry was stabilized, during the 4- to 8-week periods before fish were added. Fingerling and adult fish were stocked 1 to 2 weeks before applications of the toxicant.

The rate of detoxification of the antimycin was observed, and some of the killed fish were shipped to the Wisconsin Alumni Research Foundation for mammalian toxicity tests. Bottom fauna were sampled and quantitated. Data were obtained on water chemistry during

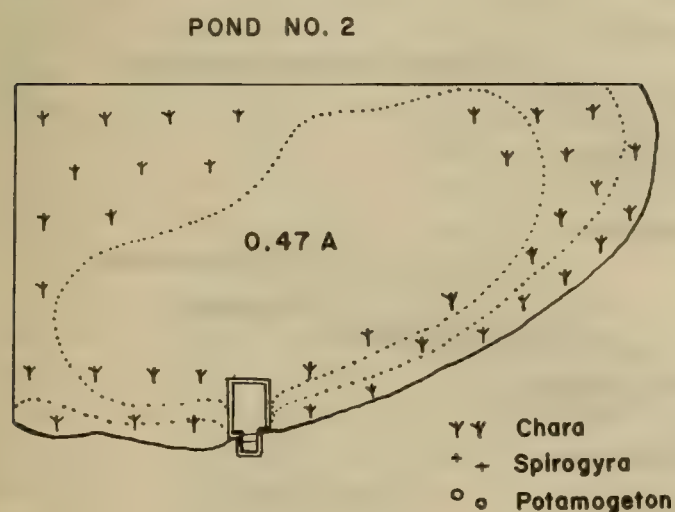
the course of tests according to standard methods (American Public Health Association et al., 1960).

Hatchery ponds.--The Wisconsin Conservation Department provided two ponds for tests at the Delafield Warmwater Fisheries Research Station in September 1963. The surface areas of ponds No. 2 and No. 5 are 0.47 and 0.78 acre respectively (fig. 2).

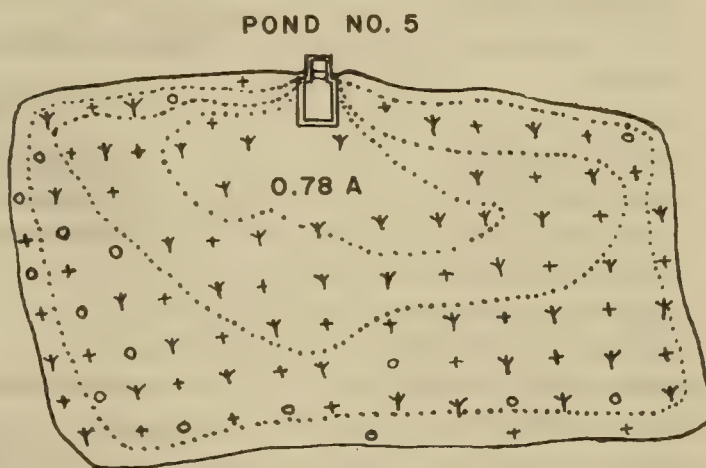
Pond No. 2 was stocked with 18 species of fish at the rate of 240 pounds per acre, and pond No. 5 with 19 species at 225 pounds per acre, 1 week before antimycin was applied. Samples of water, plankton, and bottom fauna were taken from each pond soon after the fish were stocked and again just before the ponds were drained (table 2).

TABLE 2.--Concentrations of antimycin A which caused all-or-none survival among rainbow trout and brown trout at selected water temperatures in 24 and 96 hours

Species	Number of fish	Temperature (° C)	Concentrations (p.p.b.) and survival			
			At 24 hours		At 96 hours	
			All	None	All	None
Rainbow trout....	1,829	12	0.10	0.60	0.02	0.08
Do.....	120	17	0.02	0.08	<0.02	0.04
Brown trout.....	348	12	0.10	0.40	<0.06	0.08
Do.....	120	17	0.02	0.06	<0.04	0.06



CONTOUR	ACRE- FEET
0 - 1 ft.	0.44
1 - 2 ft.	0.33
2 + ft.	0.01
TOTAL	0.78



CONTOUR	ACRE- FEET
0 - 1 ft.	0.68
1 - 2 ft.	0.63
2 - 3 ft.	0.42
3 + ft.	0.17
TOTAL	1.90

Figure 2.--Sketch of ponds No. 2 and No. 5 at the Delafield Warmwater Fisheries Research Station.

Two formulations of antimycin A were prepared for application at 10 p.p.b. Pond No. 2 received 9.72 grams of technical material in a carrier formulated by the S. B. Penick Company to make up a total volume of 300 ml. Pond No. 5 received 23.37 grams of technical material dissolved in 300 ml. of acetone as a carrier. Each aliquot was mixed with 2 gallons of water and applied to a pond surface with a hand-powered garden sprayer. The applications were made from a rowboat in late afternoon, and frequent observations were made during the next 8 hours. Observations and recovery of dead fish were made daily in the following 4 days.

RESULTS OF LABORATORY STUDIES

We found that antimycin A is toxic to the 24 species of fish tested. The toxicity varies among species and is correlated with water temperature and time. Trends in sensitivity reflect taxonomic relationships of the fishes,

and variations in susceptibility among individuals was more pronounced in some species than others. The following remarks pertain principally to the concentrations which delineate the all-or-none survival EC_0 to EC_{100} ranges, of fish at 24 or 96 hours in bioassays at 12°, 17°, or 22° C. Data are shown graphically in figures 3 and 4.

Among the 24 species, the group of fish most sensitive to antimycin A includes gizzard shad, rainbow trout, brown trout, white sucker, Iowa darter, yellow perch, and walleye. All survived exposure to 0.08 p.p.b. for 24 hours at 12° C; all perished at 0.8 p.p.b.

The group intermediate in sensitivity included northern pike, stoneroller, carp, golden shiner, fathead minnow, bigmouth buffalo, brook stickleback, green sunfish, pumpkinseed, bluegill, longear sunfish, largemouth bass, and white crappie (fig. 5). Concentrations of 0.1 and 1.6 p.p.b. defined their all-or-none survival in 24 hours at 12° C.

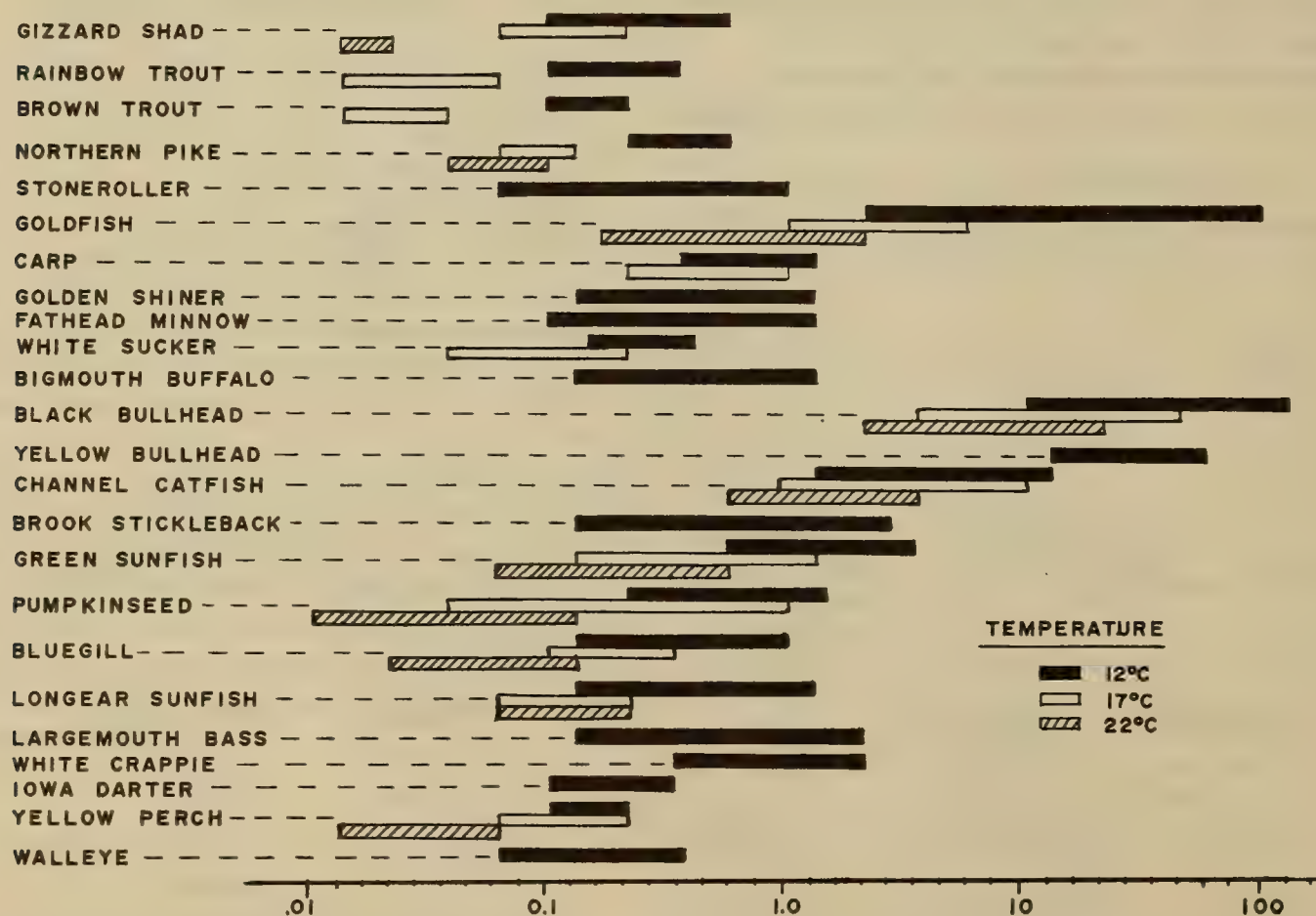


Figure 3.--The 24-hour responses of 24 fishes in the laboratory to antimycin A in p.p.b. The solid, plain, and cross hatched bars span the ranges between the EC_0 and EC_{100} at 12°, 17°, and 22° C.

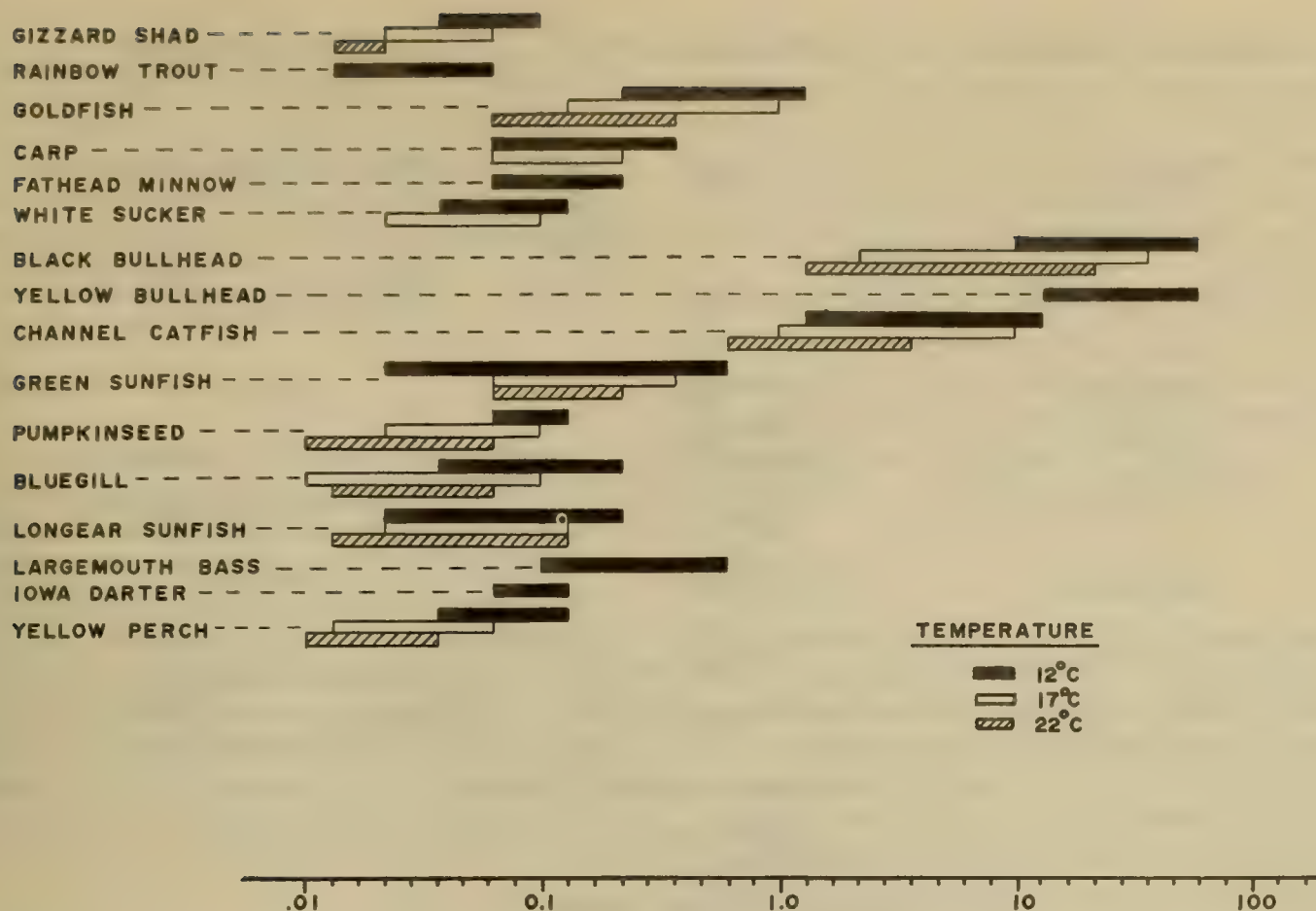


Figure 4.--The 96-hour responses of 16 fishes in the laboratory to antimycin A in p.p.b. The solid, plain, and crosshatched bars span the ranges between the EC_0 and EC_{100} at 12°, 17°, and 22° C.

The more resistant group of fish was represented by goldfish, black bullhead, yellow bullhead, and channel catfish. The concentrations required for kills in 24 hours at 12° C were 20 p.p.b. for channel catfish, 80 p.p.b. for yellow bullhead, 100 p.p.b. for goldfish, and 120 p.p.b. for black bullhead.

Increases in water temperature or duration of exposure made significant differences in the toxicity of antimycin A to fish in the three groups. For example, the toxicity to goldfish was increased tenfold at the higher temperature of 17° C. Among catfishes, the toxicity was enhanced about twofold at 17°. At the maximum temperature of 22°, the black and yellow bullheads were about 10 times as tolerant to antimycin A as goldfish, but channel catfish were only slightly more resistant.

For more detailed discussion on the toxicity of antimycin A, the species are grouped according to their respective families. The

families, in turn, are presented in order of their sensitivity to the toxicant.

Trouts

Rainbow trout and brown trout were extremely sensitive to antimycin A (table 2). At 12°, the rainbow trout succumbed to 0.6 p.p.b. in 24 hours and to 0.08 p.p.b. in 96 hours. At the same temperature, brown trout were killed by 0.4 p.p.b. in 24 hours and by 0.08 p.p.b. in 96 hours. Both species tolerated concentrations of 0.1 p.p.b. for 24 hours. In 96-hour tests, the rainbow trout survived 0.02 p.p.b. whereas brown trout withstood 0.06 p.p.b.

Herrings

At 12° C., all gizzard shad died within 24 hours upon exposure to 0.8 p.p.b. and within 96 hours at 0.1 p.p.b. (table 3). They were especially sensitive to the toxicant at 22°:

MOST SENSITIVE

INTERMEDIATE

LEAST SENSITIVE

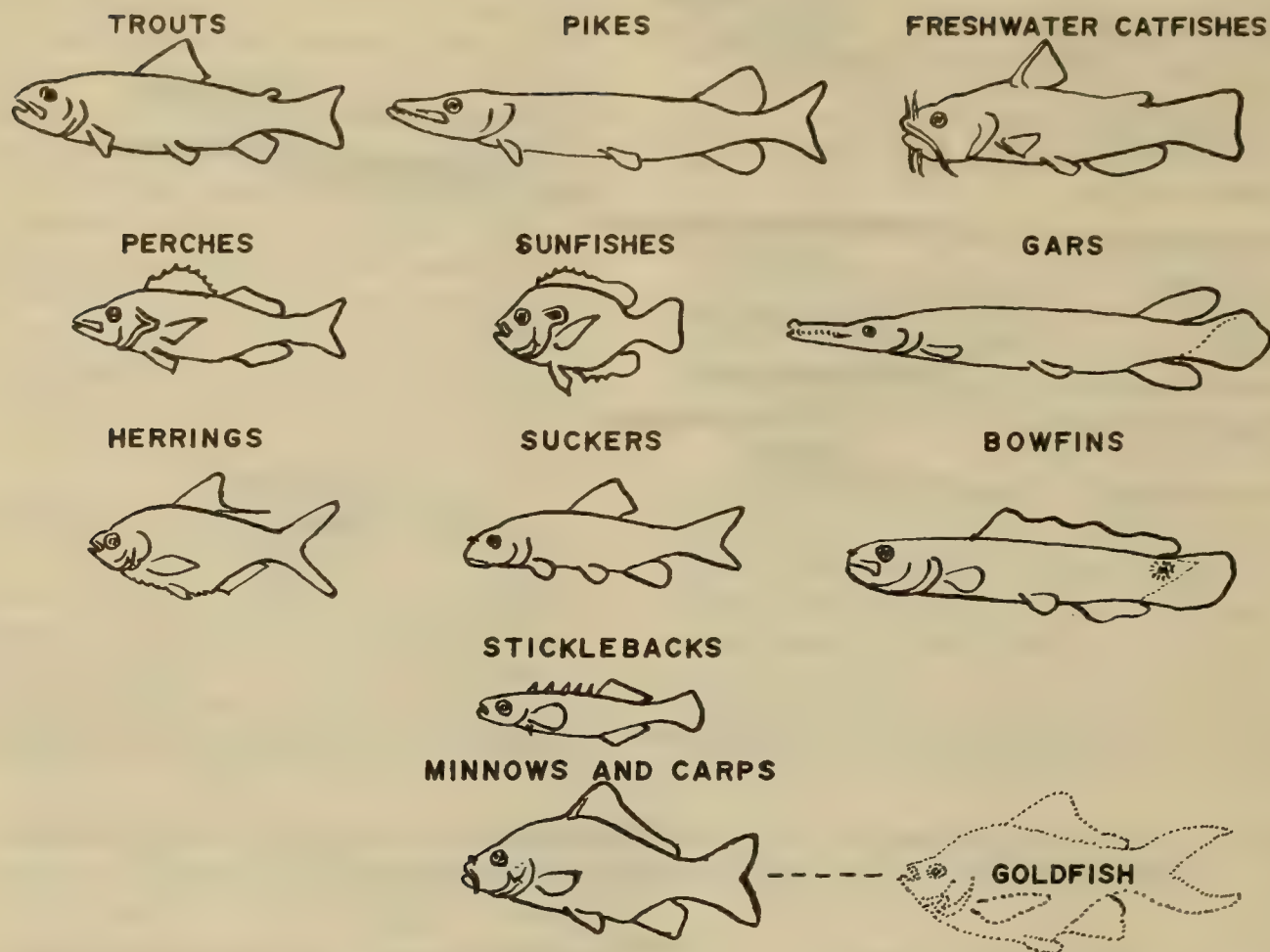


Figure 5.--The order of sensitivity of 11 families of fish to antimycin A in the laboratory and field.

concentrations of 0.04 p.p.b. caused complete kills within 24 hours, and partial kills occurred at 0.02 p.p.b. or more. It was noted that a narrow range of concentrations yielded all-or-none survival, particularly at the higher temperature and longer exposure.

Perches

The Iowa darter, yellow perch, and walleye were also very sensitive to antimycin A (table 4). All specimens in 0.08 p.p.b. at 12° for 24 hours survived, but those in 0.66 p.p.b. died. The narrow range in concentrations which caused all-or-none survival was more apparent at 22° and 96-hour exposures. Yellow perch, for example, survived 0.02 p.p.b. for 24 hours and 0.01 p.p.b. for 96 hours; they

died at 0.08 p.p.b. within 24 hours and at 0.06 p.p.b. within 96 hours.

Pikes

The fry and fingerlings of northern pike were difficult to use in bioassays because of cannibalism and rapid growth. Nevertheless, they exhibited great susceptibility to antimycin A. Complete kills were obtained in 24

TABLE 3.--Concentrations of antimycin A which caused all-or-none survival among gizzard shad at selected water temperatures in 24 and 96 hours

Number of fish	Temperature (°C.)	Concentrations (p.p.b.) and survival			
		At 24 hours		At 96 hours	
		All	None	All	None
120	12	0.10	0.80	0.06	0.10
60	17	0.08	0.40	0.04	0.08
60	22	0.02	0.04	0.02	0.04

hours by 0.8 p.p.b. at 12^o, 0.2 p.p.b. at 17^o, and 0.1 p.p.b. at 22^o. In contrast, all specimens survived 0.4 p.p.b. at 12^o, 0.08 p.p.b. at 17^o, and 0.06 p.p.b. at 22^o. Greater toxicity was detected in 48-hour exposures, but the concentrations related to all-or-none survival were not defined.

Suckers

The white sucker and bigmouth buffalo differed in their sensitivities to the toxicant, and the former was among the most susceptible fishes tested (table 5). Concentrations greater than 0.06 p.p.b. produced partial kills of white suckers at 12^o in 96 hours, and 0.22 p.p.b. caused complete kills. Even greater sensitivity was observed at 22^o. The bigmouth buffalo, on the other hand, required concentrations of antimycin A in excess of 0.4 p.p.b. for complete kills in 96 hours at 12^o.

Sunfishes

Green sunfish, pumpkinseed, bluegill, longear sunfish, largemouth bass, and white crappie were moderately sensitive to antimycin A

TABLE 4.--Concentrations of antimycin A which caused all-or-none survival among Iowa darters, yellow perch, and walleye at selected water temperatures in 24 and 96 hours

Species	Number of fish	Temperature (°C.)	Concentrations (p.p.b.) and survival			
			At 24 hours		At 96 hours	
			All	None	All	None
Iowa darters....	275	12	0.10	0.66	0.08	0.14
Yellow perch....	560	12	0.10	0.40	0.06	0.20
Do.....	504	17	0.08	0.40	0.02	0.08
Do.....	60	22	0.02	0.08	0.01	0.06
Walleye.....	20	12	0.08	0.60	--	--
Do.....	20	17	<0.08	0.10	--	--

TABLE 5.--Concentrations of antimycin A which caused all-or-none survival among white sucker and bigmouth buffalo at selected temperatures in 24 and 96 hours

Species	Number of fish	Temperature (°C.)	Concentrations (p.p.b.) and survival			
			At 24 hours		At 96 hours	
			All	None	All	None
White sucker....	810	12	0.22	0.64	0.06	0.22
Do.....	36	17	0.06	0.40	0.04	0.10
Do.....	72	22	<0.04	0.20	<0.06	0.10
Bigmouth buffalo	430	12	0.20	2.00	<0.10	0.40

(table 6). The concentrations required to cause complete kills of them at 12^o ranged from 1 to 6 p.p.b. in 24 hours and from 0.2 to 0.8 p.p.b. in 96 hours. At 22^o, killing concentrations ranged from 0.2 to 0.8 p.p.b. in 24 hours and from 0.08 to 0.4 p.p.b. in 96 hours.

The pumpkinseed and bluegill were the more sensitive of the six species, and they were followed in order of decreasing sensitivity by longear sunfish, largemouth bass, white crappie, and green sunfish.

Sticklebacks

Brook sticklebacks were moderately sensitive to antimycin A at 12^o. Concentrations of 5 p.p.b. killed all specimens within 24 hours, and partial kills occurred at concentrations greater than 0.5 p.p.b. The exposures beyond 24 hours failed to give consistent results. The condition of the fish was suspect because of difficulty in maintaining them without feeding during the longer test periods.

Minnows and carps

Stoneroller, goldfish, carp, golden shiner, and fathead minnow responded over a wide range of concentrations in an interesting pattern of susceptibility. In contrast to other families, the minnows exhibited greater variation in response between species as well as between individual specimens (table 7).

TABLE 6.--Concentrations of antimycin A which caused all-or-none survival among green sunfish, pumpkinseed, bluegill, longear sunfish, largemouth bass, and white crappie at selected water temperatures in 24 and 96 hours

Species	Number of fish	Temperature (°C.)	Concentrations (p.p.b.) and survival			
			At 24 hours		At 96 hours	
			All	None	All	None
Green sunfish....	396	12	0.80	6.00	0.04	0.80
Do.....	216	17	0.20	2.00	0.08	0.60
Do.....	30	22	0.08	0.80	0.08	0.40
Pumpkinseed.....	480	12	0.40	2.00	0.08	0.20
Do.....	120	17	0.06	1.00	0.04	0.10
Do.....	180	22	0.01	0.20	0.01	0.08
Bluegill.....	1,053	12	0.20	1.00	0.06	0.40
Do.....	360	17	0.10	0.60	0.01	0.10
Do.....	200	22	0.04	0.20	0.02	0.08
Longear sunfish..	240	12	0.20	2.00	0.04	0.40
Do.....	48	17	0.08	0.40	0.04	0.20
Do.....	48	22	0.08	0.40	0.02	0.20
Largemouth bass..	800	12	0.20	<6.00	0.10	0.80
White crappie....	180	12	0.60	>2.00	--	--

TABLE 7.--Concentrations of antimycin A which caused all-or-none survival among stoneroller, goldfish, carp, golden shiner, and fathead minnow at selected water temperatures in 24 and 96 hours

Species	Number of fish	Temperature (° C)	Concentrations (p.p.b.) and survival			
			At 24 hours		At 96 hours	
			All	None	All	None
Stoneroller.....	531	12	0.08	1.00	--	--
Goldfish.....	1,469	12	4.00	100.00	0.40	2.00
Do.....	312	17	1.00	8.00	0.20	1.00
Do.....	200	22	0.30	4.00	0.08	0.60
Carp.....	240	12	0.60	2.00	0.08	0.60
Do.....	84	17	0.40	1.00	0.08	0.40
Golden shiner....	60	12	0.20	2.00	0.05	0.60
Do.....	60	17	0.10	0.40	--	--
Do.....	40	22	0.05	0.50	--	--
Fathead minnow...	816	12	0.10	2.00	0.08	0.40
Do.....	96	17	<0.80	2.00	<0.06	0.10
Do.....	78	22	<0.10	0.80	<0.10	0.10

An outstanding highlight of the screening program was the discovery that carp are vulnerable to small concentrations of antimycin A. This prolific exotic is widely considered a most undesirable species in game-fish waters and is difficult to control with existing means.

At 12° all test carp were killed by 2 p.p.b. of antimycin in 24 hours and by 0.6 p.p.b. in 96 hours; at 17° all were killed by 1 p.p.b. in 24 hours and by 0.4 p.p.b. in 96 hours. Temperatures had less effect on toxicity to carp than to most species. There were only slight differences due to temperature in 24-hour exposures and even less at 96 hours. All carp survived 0.08 p.p.b.

The results on goldfish contrasted sharply with those on carp. In fact, the goldfish was the most tolerant of the minnows tested against antimycin A. It required 100 p.p.b. for complete kills within 24 hours at 12°, but only 2 p.p.b. were needed for kills within 96 hours. Higher temperatures contributed to greater toxicities, and all goldfish perished within 96 hours when exposed to 1 p.p.b. at 17° and 0.6 p.p.b. at 22°.

Stonerollers were among the more sensitive minnows. Concentrations of toxicant as low as 1 p.p.b. killed all specimens within 24 hours at 12°, but variations in suscepti-

bility were observed; a concentration which killed on one occasion failed on the next.

The golden shiner and fathead minnow were somewhat similar to the stoneroller in sensitivity, but all-or-none effects were delineated within a narrow range of concentrations. The golden shiners succumbed to 0.6 p.p.b. within 96 hours at 12°, and survival was noted at 0.05 p.p.b. Fathead minnows died at 0.4 p.p.b. and survived at 0.08 p.p.b.

Fresh-water catfishes

The catfishes were significantly less sensitive to antimycin A than other families (table 8). Channel catfish were more susceptible than bullheads. They survived 24-hour exposures at 12° to 2 p.p.b. but perished at 20 p.p.b. All specimens died at 6 p.p.b. in 96-hour tests at 22°.

The black bullhead was the more tolerant to the toxicant, and the yellow bullhead was only slightly less so. Concentrations of 120 and 100 p.p.b. respectively were required for complete kills in 24 hours at 12°. These concentrations are more than 100 times greater than those needed to kill fish of the most sensitive families.

The bullheads were affected by somewhat smaller quantities of chemical at 17°. Nevertheless, black bullheads tolerated 4 p.p.b. for 24 hours at 22°, and all died at 40 p.p.b.

TABLE 8.--Concentrations of antimycin A which caused all-or-none survival among black bullhead, yellow bullhead, and channel catfish at selected water temperatures in 24 hours and 96 hours

Species	Number of fish	Temperature (° C)	Concentrations (p.p.b.) and survival			
			At 24 hours		At 96 hours	
			All	None	All	None
Black bullhead...	848	12	10.0	120.0	10.0	80.0
Do.....	120	17	6.0	60.0	4.0	40.0
Do.....	120	22	4.0	40.0	2.0	40.0
Yellow bullhead..	192	12	20.0	80.0	20.0	80.0
Do.....	84	17	<10.0	60.0	--	--
Channel catfish..	120	12	2.0	20.0	2.0	20.0
Do.....	120	17	1.0	10.0	1.0	10.0
Do.....	180	22	0.8	6.0	0.8	6.0

Other animals

Four hundred water fleas were used in trials with antimycin A. At 12° C., specimens survived 1 and 0.5 p.p.b., but died in 100 p.p.b. in 24 hours and in 10 p.p.b. in 48 hours. Their susceptibility increased with temperature. At 22°, they survived 0.1 p.p.b., but died in 10 p.p.b. in 24 hours and in 0.5 p.p.b. in 48 hours.

There were no mortalities among 20 crayfish exposed to 10 p.p.b. of toxicant at 12° for 96 hours.

Tests with 120 damselfly nymphs disclosed that the insects were relatively tolerant to antimycin A. At 12°, specimens survived 100 and 50 p.p.b. for 24 and 48 hours respectively, and 1,000 and 500 p.p.b. were required to kill them in the same time periods. At 22°, they survived 50 and 10 p.p.b., but died at 500 and 100 p.p.b. in 24 and 48 hours. The observations were not continued to 96 hours because high mortalities began to occur among controls.

Ninety-six tiger salamanders were exposed to antimycin A at 12°. Specimens survived 80 p.p.b. for 96 hours, but were killed by 600 p.p.b.

Among the 40 bullfrog tadpoles tested for 24 hours at 12°, the individuals exposed to 20 p.p.b. of toxicant survived whereas those subjected to 40 p.p.b. perished.

RESULTS OF FIELD STUDIES

Tests in wading pools

Results in 1962.--Some preliminary bioassays were conducted in 18 pools in July and October, to determine the utility of the pools as bioassay vessels and to yield information on the performance of antimycin A outdoors. A shortage of toxicant limited the scope of the trials, and a scarcity of fish of desirable species, sizes, and condition affected their validity. A number of the species were wild fish which later proved to be unsatisfactory test animals because of variable sizes, heavy parasitism, and poor condition.

The wading pools worked well as bioassay vessels. Fish, invertebrates, and plants did well in the test units and controls. There were some differences in the quantity of plankton and aquatic vegetation in the sand- and loam-bottom units because the latter were more fertile. The abundance of plants, we believe, contributed to increases in pH and alterations of alkalinity, and these in turn influenced the efficacy of antimycin A.

Goldfish, golden shiner, black bullhead, bluegill, largemouth bass, and yellow perch were exposed to 5 and 10 p.p.b. of toxicant in July. Most of them survived in the sand pools. The mortality was greater in the loam pools, especially at 10 p.p.b., but in no instance did it reach 100 percent. The black bullheads exhibited high tolerance to the toxicant in all pools.

Another series of tests was made in October with higher concentrations against rainbow trout, goldfish, golden shiner, bluntnose minnow, yellow bullhead, green sunfish, and yellow perch (table 9). The pH values in the pools at the time ranged from 7.5 to 9.9. Ninety to 100 percent of the trout, golden shiner, bluntnose minnow, green sunfish, and perch, and 60 percent of the goldfish were killed by 20 p.p.b. over sand and loam bottoms. At 40 p.p.b., there was very low survival among the trout, goldfish, and sunfish, but nearly complete survival of bullheads.

There appeared to be rapid degradation and detoxification of antimycin in the pools within 24 to 96 hours, depending on the initial concentration and the pH. Small numbers of goldfish, golden shiner, bluntnose minnow, bluegill, and largemouth bass were stocked later in pools in which antimycin A had been present for 24 to 72 hours. No more than half of the golden shiners and bluegills perished within the following 2 days.

Results in 1963.--The plants, plankton, and bottom fauna were permitted to develop in the pools for 2 months before toxicity trials. In July, acetone solutions of antimycin A were tested at 10, 20, 40, and 80 p.p.b. against eight species of fish of various sizes (tables 10, 11, 12, and 13). Golden shiners, bluegills, largemouth bass, and yellow perch were the more

TABLE 9.--Toxicity of antimycin A at 20 and 40 p.p.b. on adult and fingerling fish in sand- and loam-bottom pools

[Mortalities are cumulative by observation periods]

Species of fish	Type of bottom	Antimycin A at 20 p.p.b.					Antimycin A at 40 p.p.b.				
		Number of fish	Number dead in (hours)--				Number of fish	Number dead in (hours)--			
			24	48	96	336		24	48	96	336
Adults:											
Rainbow trout.....	sand	20	16	16	17	19	20	16	17	18	20
Do.....	loam	20	8	12	17	18	20	14	16	17	19
Yellow bullhead.....	sand	40	0	0	0	1	40	0	0	0	1
Do.....	loam	40	0	0	0	3	40	0	0	0	0
Green sunfish.....	sand	40	0	0	7	37	40	1	1	12	40
Do.....	loam	40	0	0	5	39	40	2	2	11	39
Fingerlings:											
Goldfish.....	sand	40	1	11	17	25	40	3	21	37	37
Do.....	loam	40	1	7	16	26	40	14	40	--	--
Golden shiner.....	sand	40	35	39	40	--	40	40	--	--	--
Do.....	loam	40	37	40	--	--	40	40	--	--	--
Bluntnose minnow.....	sand	40	33	39	40	--	40	40	--	--	--
Do.....	loam	40	31	39	40	--	40	40	--	--	--
Yellow perch.....	sand	40	40	--	--	--	40	40	--	--	--
Do.....	loam	40	40	--	--	--	40	40	--	--	--

TABLE 10.--Numbers and sizes of fish exposed to antimycin A in wading pools in July 1963

Species	Total number	Average weight (grams)
Goldfish.....	180	1.0
Carp.....	180	2.2
Golden shiner.....	108	1.0
Black bullhead.....	180	2.0
Do.....	180	18.0
Green sunfish.....	144	2.7
Bluegill.....	270	0.8
Do.....	180	22.0
Largemouth bass.....	144	1.5
Yellow perch.....	270	2.5

sensitive, and they were killed by 10 p.p.b. within 48 hours over sand and loam bottoms. Carp and green sunfish perished at 10 p.p.b. within 24 hours over loam bottoms and at 20 p.p.b. in the same time over sand soil. Some lots of goldfish died at 20 p.p.b., but all succumbed at 40 p.p.b. A concentration of 80 p.p.b. killed all fingerling and adult black bullheads within 48 hours over sand bottom but only one-half of them over loam.

The green sunfish and yellow perch appeared at the surface of pools within 2 hours

TABLE 11.--Toxicity of antimycin A at 10 and 20 p.p.b. on adult and fingerling fish in sand- and loam-bottom wading pools

[Mortalities are cumulative by observation period]

Species	Type of bottom	Antimycin A at 10 p.p.b.					Antimycin A at 20 p.p.b.				
		Number of fish	Number dead in (hours)--				Number of fish	Number dead in (hours)--			
			24	48	96	480		24	48	96	480
Adults:											
Black bullhead.....	sand	20	0	0	0	2	20	0	0	0	0
Do.....	loam	20	0	0	0	1	20	0	0	0	3
Bluegill.....	sand	20	17	20	--	--	20	20	--	--	--
Do.....	loam	20	20	--	--	--	20	20	--	--	--
Fingerlings:											
Goldfish.....	sand	20	6	6	6	6	20	20	--	--	--
Do.....	loam	20	7	7	7	7	20	10	10	10	10
Carp.....	sand	20	14	14	14	14	20	20	--	--	--
Do.....	loam	20	20	--	--	--	20	14	14	14	14
Golden shiner.....	sand	14	14	--	--	--	14	14	--	--	--
Do.....	loam	14	14	--	--	--	14	14	--	--	--
Black bullhead.....	sand	20	0	0	0	0	20	0	0	0	0
Do.....	loam	20	0	0	0	0	20	0	0	0	0
Green sunfish.....	sand	16	15	15	15	15	16	16	--	--	--
Do.....	loam	16	16	--	--	--	16	16	--	--	--
Bluegill.....	sand	40	40	--	--	--	40	40	--	--	--
Do.....	loam	40	40	--	--	--	40	40	--	--	--
Largemouth bass.....	sand	16	16	--	--	--	16	16	--	--	--
Do.....	loam	16	16	--	--	--	16	16	--	--	--
Yellow perch.....	sand	20	20	--	--	--	20	20	--	--	--
Do.....	loam	20	18	20	--	--	20	20	--	--	--

TABLE 12.--Toxicity of antimycin A at 40 and 80 p.p.b. on adult and fingerling fish in sand- and loam-bottom wading pools

[Mortalities are cumulative by observation period]

Species	Type of bottom	Antimycin A at 40 p.p.b.					Antimycin A at 80 p.p.b.				
		Number of fish	Number dead in (hours)--				Number of fish	Number dead in (hours)--			
			24	48	96	480		24	48	96	480
Adults:											
Black bullhead.....	sand	20	0	0	0	2	20	6	20	--	--
Do.....	loam	20	0	0	0	1	20	1	2	2	3
Bluegill.....	sand	20	20	--	--	--	20	20	--	--	--
Do.....	loam	20	20	--	--	--	20	20	--	--	--
Fingerlings:											
Goldfish.....	sand	20	20	--	--	--	20	20	--	--	--
Do.....	loam	20	20	--	--	--	20	20	--	--	--
Carp.....	sand	20	20	--	--	--	20	20	--	--	--
Do.....	loam	20	20	--	--	--	20	20	--	--	--
Golden shiner.....	sand	14	14	--	--	--	14	14	--	--	--
Do.....	loam	14	14	--	--	--	14	14	--	--	--
Black bullhead.....	sand	20	0	0	0	0	20	20	--	--	--
Do.....	loam	20	0	0	0	0	20	11	11	11	11
Green sunfish.....	sand	16	16	--	--	--	16	16	--	--	--
Do.....	loam	16	16	--	--	--	16	16	--	--	--
Bluegill.....	sand	40	40	--	--	--	40	40	--	--	--
Do.....	loam	40	40	--	--	--	40	40	--	--	--
Largemouth bass.....	sand	16	16	--	--	--	16	16	--	--	--
Do.....	loam	16	16	--	--	--	16	16	--	--	--
Yellow perch.....	sand	20	20	--	--	--	20	20	--	--	--
Do.....	loam	20	18	20	--	--	20	14	20	--	--

TABLE 13.--Average values of analyses made on water from sand- and loam-bottom wading pools before and after applications of antimycin A in July 1963

Item	Unit of measurement	Sand		Loam	
		Before	After	Before	After
Temperature.....	°C	23	25	23	27
Resistivity.....	at 20°C	2803	2864	3037	3052
Dissolved oxygen.....	p.p.m.O ₂	8.7	9.1	9.7	9.7
Carbon dioxide.....	p.p.m.CO ₂	0.0	0.0	0.0	0.0
Hydrogen ion.....	pH	8.8	9.1	8.8	9.2
Total alkalinity.....	p.p.m.CaCO ₃	204.4	181.2	198.2	183.7
(as phenolphthalein).....		(10.7)	(11.5)	(14.6)	(14.3)
(as methyl orange).....		(193.7)	(169.7)	(183.6)	(169.4)
Total hardness.....	p.p.m.CaCO ₃	211.8	176.0	210.6	182.0
Calcium hardness.....	p.p.m.CaCO ₃	53.6	47.9	60.0	53.6
Total iron.....	p.p.m.Fe ⁰	0.0	0.0	0.0	0.0
Sulfate ion.....	p.p.m.SO ₄	25.8	13.4	18.3	11.1
Total phosphorus.....	p.p.m.PO ₄	0.059	0.071	0.082	0.106
Ammonia nitrogen.....	p.p.m.NH ₃	0.399	0.730	0.710	1.100
Nitrite nitrogen.....	p.p.m.NO ₂	0.006	0.013	0.005	0.018
Nitrate nitrogen.....	p.p.m.NO ₃	0.117	0.191	0.154	0.220
Chloride ion.....	p.p.m.CL	10.5	16.5	10.2	15.1

after exposure to the toxicant, and they exhibited a narcosislike condition. They showed little response to motion stimulus or handling with a dip net. Some of the larger bullheads behaved as if in distress and were subject to development of an unidentified funguslike condition on the body prior to death.

The trials in October included two formulations of antimycin. One was a solution in acetone, and the other an emulsifiable concentrate, applied to pools at 1, 5, 10, and 100 p.p.b. against 10 species of fish. The pH values at the time in all pools were about 10, and the antimycin A degraded so rapidly that most fish escaped toxic effects (table 14). The

TABLE 14.--Average values of analyses made on water from sand- and loam-bottom wading pools before and after applications of antimycin A in October 1963

Item	Unit of measurement	Sand		Loam	
		Before	After	Before	After
Temperature.....	°C	16	16	16	15
Resistivity.....	at 20°C	3561	3431	3439	3396
Dissolved oxygen.....	p.p.m.O ₂	10.0	9.6	10.0	9.5
Carbon dioxide.....	p.p.m.CO ₂	0.0	0.0	0.0	0.0
Hydrogen ion.....	pH	10.0	10.0	10.0	9.8
Total alkalinity.....	p.p.m.CaCO ₃	114.0	107.0	127.0	121.0
(as phenolphthalein).....		(29.0)	(27.5)	(36.0)	(34.0)
(as methyl orange).....		(85.0)	(79.5)	(91.0)	(88.0)
Total hardness.....	p.p.m.CaCO ₃	143.0	148.0	155.0	154.0
Calcium hardness.....	p.p.m.CaCO ₃	27.4	33.0	38.0	35.0
Total iron.....	p.p.m.Fe ⁰	0.025	0.026	0.036	0.028
Sulfate ion.....	p.p.m.SO ₄	17.8	15.3	18.0	14.0
Total phosphorus.....	p.p.m.PO ₄	0.090	0.084	0.043	0.035
Ammonia nitrogen.....	p.p.m.NH ₃	0.25	0.270	0.000	0.550
Nitrite nitrogen.....	p.p.m.NO ₂	0.0	0.0	0.0	0.0
Nitrate nitrogen.....	p.p.m.NO ₃	0.0	0.0	0.0	0.0
Chloride ion.....	p.p.m.Cl	12.6	15.25	11.0	13.6

exceptions were those individuals exposed to 100 p.p.b. It appeared that the acetone solution of toxicant deteriorated sooner than the other preparation.

Of the 10 species of fish, 7 species succumbed totally to 100 p.p.b. of acetone-antimycin A, and 9 species to the emulsifiable formulation, within 24 hours over sand bottoms; only carp, fathead minnow, bluegill, longear sunfish, and yellow perch died over loam bottoms. The black bullhead was the sole survivor of 100 p.p.b. over both bottom types. Neither preparation of toxicant caused 100-percent kills of any species within 96 hours at 5 or 10 p.p.b.

In general, most of the susceptible fish showed signs of distress within a short time after exposure, and many came to the surface of the pools. The length of time which elapsed before death varied with the species and water temperature, and ranged from a few hours to several days. It is significant that all specimens which displayed symptoms of distress eventually died. This suggests that the action of the toxicant on fish is irreversible.

There were no grossly toxic effects by antimycin A on the plankton, bottom fauna, or aquatic plants during the course of the July and October trials. For example, in the four pools which received 20 p.p.b. of antimycin A in July, the average quantity of plankton was 0.0036 cc./l. (range: 0.0020 to 0.0044) before treatment and 0.0040 cc./l. (range: 0.0033 to 0.0061) at 20 days after treatment. The quantities in two control pools were 0.0047 and 0.0089 cc./l. during pretreatment sampling and 0.0022 and 0.0044 cc./l. during post-treatment sampling.

Tests in hatchery ponds

There appeared to be a more rapid response of fish to the antimycin A which was formulated with an emulsifiable concentrate than with acetone. With the former preparation in pond No. 2, fish surfaced within 4 to 6 hours after application, whereas in pond No. 5 there were no comparable effects for another 10 hours. By the end of the first full

day, we saw no significant differences in the effects produced by the two formulations. Table 15 gives before and after water analyses for the two ponds.

Northern pike were the first fish to exhibit distress. They surfaced and appeared to be in a state of narcosis which was followed by complete locomotor ataxia. The rainbow trout, white suckers, carp, walleye, and sunfishes followed in order with similar symptoms. The great majority of specimens were dead within 48 hours (tables 16 and 17). It is noteworthy that goldfish--a species which was relatively

TABLE 15.--Analyses of water from ponds No. 2 and No. 5 at Delafield Warmwater Fisheries Research Station before and after applications of antimycin A in September 1963

Item	Unit of measurement	Pond No. 2		Pond No. 5	
		Before	After	Before	After
Temperature.....	°C	21	15	21	17
Resistivity.....	at 20°C	2550	2600	2525	2600
Dissolved oxygen.....	p.p.m.O ₂	6.7	6.9	7.5	8.2
Carbon dioxide.....	p.p.m.CO ₂	3.4	0.0	2.0	0.0
Hydrogen ion.....	pH	8.0	8.4	8.9	8.5
Total alkalinity.....	p.p.m.CaCO ₃	210.0	202.0	201.1	189.5
(as phenolphthalein).....		(0.0)	(0.0)	(8.8)	(0.0)
(as methyl orange).....		(210.0)	(202.0)	(192.3)	(189.5)
Total hardness.....	p.p.m.CaCO ₃	213.0	220.0	202.0	208.0
Calcium hardness.....	p.p.m.CaCO ₃	77.0	82.0	80.0	75.0
Manganese.....	p.p.m.Mn ⁰	0.0	0.0	0.0	0.0
Total iron.....	p.p.m.Fe ⁰	0.00	0.05	0.00	0.13
Sulfate ion.....	p.p.m.SO ₄	44.3	38.0	39.0	35.0
Total phosphorus.....	p.p.m.PO ₄	1.40	0.10	0.50	0.15
Ammonia nitrogen.....	p.p.m.NH ₃	0.20	0.19	0.18	0.38
Nitrite nitrogen.....	p.p.m.NO ₂	0.0	0.0	0.0	0.0
Nitrate nitrogen.....	p.p.m.NO ₃	0.07	0.50	0.07	0.43
Chloride ion.....	p.p.m.CL	--	14.5	--	15.0

TABLE 16.--Effects of 10 p.p.b. of antimycin A in emulsifiable concentrate on 18 species of fish in pond No. 2

Species	Total fish stocked	Average length (inches)	Average weight (grams)	Number of fish dead at (hours)--			
				24	48	96	480
Longnose gar.....	3	25.6	658	0	0	0	0
Bowfin.....	1	16.8	545	0	0	0	0
Rainbow trout.....	312	4.0	82	312	--	--	--
Northern pike.....	7	17.8	713	5	5	5	7
Goldfish.....	740	2.4	9	740	--	--	--
Carp.....	18	15.3	1,126	17	18	--	--
White sucker.....	4	15.1	554	3	3	3	4
Black bullhead....	600	3.8	18	0	0	0	0
Yellow bullhead...	4	8.3	168	0	0	0	0
Brown bullhead....	1	4.2	50	0	0	0	0
Rock bass.....	1	8.0	136	1	--	--	--
Green sunfish.....	3	3.8	14	0	0	1	3
Pumpkinseed.....	13	4.6	41	11	12	13	--
Bluegill.....	27	6.1	68	21	21	22	27
Black crappie.....	7	8.3	95	5	5	5	7
Largemouth bass...	4	15.4	795	1	4	--	--
Hybrid sunfish....	1,400	1.7	9	1,400	--	--	--
Walleye.....	1	13.5	318	1	--	--	--

TABLE 17.--Effects of 10 p.p.b. of antimycin A in acetone solution on 19 species of fish in pond No. 5

Species	Total fish stocked	Average length (inches)	Average weight (grams)	Number of fish dead at (hours)--			
				24	48	96	480
Longnose gar.....	3	24.6	395	0	0	2	2
Bowfin.....	1	21.8	1,771	0	0	0	0
Rainbow trout.....	470	4.1	86	470	--	--	--
Northern pike.....	8	19.1	976	6	6	8	--
Goldfish.....	1,400	2.7	9	1,400	--	--	--
Carp.....	27	15.3	1,112	26	27	--	--
White sucker.....	6	15.7	636	5	5	5	6
Black bullhead....	875	3.7	14	0	2	3	157
Yellow bullhead...	1	5.7	59	0	0	0	0
Brown bullhead....	6	11.4	377	0	0	0	1
Rock bass.....	1	8.2	136	0	0	0	1
Green sunfish.....	4	4.2	23	0	1	2	4
Pumpkinseed.....	24	4.6	32	2	12	12	24
Bluegill.....	43	6.4	86	19	28	36	43
Black crappie....	9	8.8	136	1	4	4	9
Largemouth bass....	5	12.8	477	0	2	4	5
Hybrid sunfish....	2,055	1.7	9	2,055	--	--	--
Walleye.....	2	13.0	386	2	--	--	--
Drum.....	1	11.6	272	0	0	1	--

tolerant to antimycin A in the laboratory--died in both ponds within 24 hours.

The longnose gar, bowfin, black bullhead, yellow bullhead, and brown bullhead were the only species which were not affected greatly by the toxicant at 10 p.p.b. Seventy percent of them were recaptured alive when the ponds were drained after 20 days.

The detoxification of antimycin A was monitored throughout the first 96 hours. It occurred within 72 hours after application, and fish placed in live cages after this time survived until the ponds were drained.

Plankton was sampled in both ponds during the experimental period. In pond No. 2, the pretreatment quantity was 0.018 cc./l and the posttreatment quantity was 0.047 cc./l. Pond No. 5 had pretreatment and posttreatment quantities of 0.0035 and 0.039 cc./l. None of the relatively minor changes was attributed to the toxicant. Also, there were no observable changes in the aquatic plants in the ponds.

Pretreatment and posttreatment samples of bottom fauna were taken. We concluded that antimycin A was nontoxic to the 15 taxonomic groups which were represented in both ponds because there were no significant changes in species composition or numerical abundance (table 18). The midges were the more numerous in all samples, and they increased by 55 to 65 percent during the experimental period. The nymphs of mayflies, dragonflies, and

TABLE 18.--Abundance of bottom fauna in ponds No. 2 and No. 5 before and after treatment with 10 p.p.b. of antimycin A

[Each collection consisted of 16 one-square foot samples

Organism	Average number per square foot			
	Pond No. 2		Pond No. 5	
	Sept. 23	Oct. 15	Sept. 17	Oct. 14
Horsehair worm (Nematomorpha)....	10.7	0.7	1.0	1.0
Aquatic earthworm (Oligochaeta).....	0.0	0.0	34.5	3.0
Leech (Hirundinea).....	0.0	1.3	5.2	1.0
Scuds (Amphipoda).....	4.7	2.0	17.5	39.0
Mayflies (Ephemeroptera)....	9.0	145.3	6.8	6.5
Damselflies (Zygoptera).....	1.7	2.0	6.8	7.8
Dragonflies (Anisoptera).....	0.0	0.7	0.5	0.5
Waterbugs (Hemiptera).....	0.0	2.7	1.0	1.5
Caddisflies (Trichoptera).....	0.7	2.0	0.2	0.0
Water beetles (Coleoptera).....	2.7	18.0	5.0	3.5
Mosquitoes (Culicidae).....	0.0	0.0	0.8	0.0
Midges (Tendipedidae)....	209.7	388.0	269.5	422.0
Biting midges (Ceratopogonidae)..	1.3	0.0	2.8	1.0
Soldierflies (Stratiomyidae)...	0.3	0.0	0.0	0.0
Snails (Gastropoda).....	4.0	30.7	72.8	30.5
Total.....	244.8	593.4	424.4	517.3

damselflies were also more abundant in the posttreatment samples.

Care was taken to note any gross effects of the toxicant on frogs, salamanders, and turtles, but there were none.

Discussion of field studies

There was a lack of consistency in the performance of antimycin A in sand- and loam-bottom pools in July and October, 1962 and 1963, and in the hatchery ponds. The cause, we believe, was the chemistry of the waters and particularly the presence of the hydroxyl ion.

An alkaline shift occurred in the wading pools as the plant biomass increased. The relatively hard, well water which was used to fill the pools was gradually softened because of the decrease in calcium. There was a shift from bicarbonate (methyl orange alkalinity) to free hydroxide (phenolphthalein alkalinity). The measure of the acid-base shift was pH which rose from 7.5 upward to 10 or more. Diurnal fluctuations of several pH units are not uncommon in ponds, and the pH in wading pools ranged accordingly between morning and afternoon.

The highest pH values were observed in late afternoon in the presence of abundant plants and sunshine. In this situation, the

hydroxyl ions appear, and often they are not checked by buffering salts. Magnesium prevails as calcium ions are removed from solution, and the result is the sort of alkaline shift observed in softer waters.

We assume that the relative success of the toxicity trials in hatchery ponds was due in large part to the fact that the water had high buffer capacity and little reserve alkalinity in the form of hydroxide. Thus, the antimycin A was not subject to immediate detoxification by action of free hydroxide, and the 10 p.p.b. were effective in killing fish.

In contrast, the poorer results obtained in the wading pools reflected the greater concentrations of free hydroxide present. In July 1963, the pools had approximately the same pH and total alkalinity as the hatchery ponds, but there was more free hydroxide present. Therefore, the degradation of the toxicant was more rapid, and 20 to 40 p.p.b. were needed to kill fish.

The contrast was heightened by results in October 1963. The water was much softer and lower in buffer capacity, and there was even more free hydroxide present. The pH ranged up to 10. Under these conditions, there was almost immediate detoxification of the antimycin, and only partial fish kills were obtained at 100 p.p.b.

CONCLUSIONS

Antimycin A is a powerful toxicant to fresh-water fish. We observed the responses of many specimens to concentrations which ranged from 0.01 to 120 p.p.b. Among them, the carp--a most undesirable fish in many waters--proved vulnerable to small concentrations and short exposures at cool and warm temperatures. Other fishes which at times may be undesirable, such as goldfish, white suckers, green sunfish, and pumpkinseeds, were also killed.

The sensitivities to antimycin A varied among species, and they were correlated with temperature and duration of exposure. The tests in the laboratory at 12⁰, 17⁰, and 22⁰ C.

indicated that smaller quantities of toxicant or shorter exposures produced kills of fish in warmer waters, but the results at 12⁰ were nonetheless satisfactory.

There were three general degrees of sensitivity detected among the 24 species of fish in the laboratory and a similar order among the 25 species used in outdoor trials. Indicative of the extremes in response, gizzard shad perished at 0.04 p.p.b. of toxicant whereas black bullheads survived 100 p.p.b. There also appeared to be a tendency for sensitivities to follow family lines, but species in the nine families tested exhibited great variations in susceptibility. For example, fingerling carp in the laboratory died within 24 hours upon exposure to 0.6 p.p.b. at 12⁰, but up to 100 p.p.b. were required for complete kills of goldfish.

Observations in the laboratory and field demonstrated that antimycin A was less toxic to other animals. Water fleas were killed by 100 p.p.b. in 24 hours at 12⁰, but their susceptibility increased at warmer temperatures or longer exposures. Crayfish were not harmed by 10 p.p.b. over 96 hours, and damselfly nymphs survived 50 p.p.b. for 48 hours. Tiger salamanders survived 80 p.p.b. for 96 hours at 12⁰, and bullfrog tadpoles were unharmed by 20 p.p.b. for 24 hours.

The plankton in wading pools and hatchery ponds was not significantly affected during experiments, and there was no gross evidence of toxicity to filamentous algae, and submersed and emergent plants. No deleterious effects were detected on the composition, numbers, and growth of bottom fauna in hatchery ponds.

Antimycin A degraded rapidly in water, and detoxification was complete within 24 to 96 hours under field conditions. The rate of molecular breakdown was accelerated sharply in the presence of free hydroxide, and this suggests a possibility for artificial detoxification. Bioassays with fish following the degradation of the toxicant revealed an absence of harmful residues in water.

Further investigation on antimycin A as a fish toxicant is warranted in the laboratory

and field. Studies are contemplated or in progress at the Fish Control Laboratories on its performance against various life stages of fish from egg to adult; against additional species; on minimum killing concentrations and exposures; in waters of various chemistries; and at cold and warm temperatures. Appropriate formulations for standing and flowing waters are desirable. Further attention must also be given to the effects of the toxicant on other aquatic organisms. The factors in water which contribute to degradation of the toxicant deserve study, and the nature and fate of residues require definition. Also--and depending on adequate supplies of toxicant--many, and more comprehensive, trials in the field are needed for full and fair evaluation of this material which has potential value in fishery management and research.

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FISH CONTROL

PURPOSE	METHODS				BIOLOGICAL AGENTS
	RADIANT ENERGY - LIGHT, HEAT, ELECTRICITY, SOUND	PHYSICAL AND MECHANICAL	CHEMICAL AND BIOCHEMICAL	GENETICS	
INDUCE SPAWNING	Electric shock Controlled light Sound stimulant	Hydraulic control	Hormones	Select for season and duration of spawning	Plant introductions
PREVENT SPAWNING	Electric shock Radiation Controlled light Sound depressant		Sterilants Hormones	Select for low fertility	Plant introduction or removal
IMPROVE GROWTH, VIGOR, FECUNDITY, DISEASE RESISTANCE	Light control			Brood stock selection Select for wildness Select for rapid growth Select for disease resistance	
TRANSPORT	Electro-narcosis	Aeration Hydraulic control Temperature control	Anesthetics Sedatives Decontaminants Diet manipulation		
PREVENT OSMOTIC SHOCK		Acclimatization	Osmoregulatory compounds	Select for adaptability	
PREVENT ENTRY	Electrical barrier	Barrier Hydraulic manipulation	Repellants		
RESTRICT MOVEMENT	Electrical array	Hydraulic manipulation	Attractants Repellants	Select for non-migratory strain	
SELECTIVE REDUCTION	Pulsed current	Water level manipulation Gear development	Selective toxicants		Selective infectious disease Selective parasites Predator introduction Competitor introduction
FACILITATE CAPTURE	Sonic attractant Electrical guiding array	Water level manipulation Gear development	Attractants	Select for catchability	
ERADICATION	Lethal current	Gear development	Lethal compounds		

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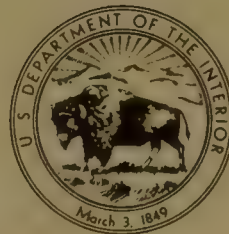
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INVESTIGATIONS IN FISH CONTROL

- 3. Minimum Lethal Levels of Toxaphene as a Piscicide in North Dakota Lakes**
- 4. Effects of Toxaphene on Plankton and Aquatic Invertebrates in North Dakota Lakes**
- 5. Growth Rates of Yellow Perch in Two North Dakota Lakes After Population Reduction with Toxaphene**
- 6. Mortality of Some Species of Fish to Toxaphene at Three Temperatures**
- 7. Treatment of East Bay, Alger County, Michigan with Toxaphene for Control of Sea Lampreys**
- 8. Effects of Toxaphene on Fishes and Bottom Fauna of Big Kitoi Creek, Afognak Island, Alaska**



United States Department of the Interior
Fish and Wildlife Service
Bureau of Sport Fisheries and Wildlife

INVESTIGATIONS IN FISH CONTROL

Investigations in Fish Control, published by the Bureau of Sport Fisheries and Wildlife, include reports on the results of work at the Bureau's Fish Control Laboratories at La Crosse, Wis., and Warm Springs, Ga., and reports of other studies related to that work. Though each report is regarded as a separate publication, several may be issued under a single cover, for economy.

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Fish Control Laboratories
Bureau of Sport Fisheries and Wildlife
U. S. Department of the Interior
P. O. Box 862
La Crosse, Wisconsin 54602

INVESTIGATIONS IN FISH CONTROL

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United States Department of the Interior, Stewart L. Udall, *Secretary*
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Fish and Wildlife Service, Clarence F. Pautzke, *Commissioner*
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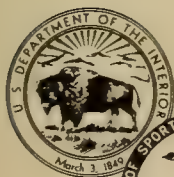
NOTE

Toxaphene has never been registered for use as a piscicide. The Bureau of Sport Fisheries and Wildlife has discontinued management use of toxaphene at least until completion of definitive studies on detoxification in relation to water quality, residual toxicities, and short-term effects of its use on aquatic organisms. These six papers on toxaphene and its effects are published as contributions to an understanding of the chemical and its use in fish management.

INVESTIGATIONS IN FISH CONTROL

**3. Minimum Lethal Levels of Toxaphene as a Piscicide
in North Dakota Lakes**

By Dale L. Henegar
Chief, Fish Management Division
North Dakota Game and Fish Department



U.S. DEPARTMENT OF THE INTERIOR
Fish and Wildlife Service
Bureau of Sport Fisheries and Wildlife
Resource Publication 7
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MINIMUM LETHAL LEVELS OF TOXAPHENE AS A PISCICIDE IN NORTH DAKOTA LAKES

By Dale L. Henegar, Chief, Fish Management Division
North Dakota Game and Fish Department

Abstract.--To determine minimum levels of toxaphene lethal to fishes in prairie lakes and reservoirs, 16 North Dakota lakes, ranging from 6.3 to 915 acres, were treated in 1959 and 1960 with concentrations of toxaphene ranging from 0.005 to 0.035 p.p.m. Physical and chemical studies were made of each area, hydrological maps were prepared, and test netting was carried out before and after treatment; application methods and equipment were according to North Dakota Game and Fish Department regulations. Mortality after treatment varied from incomplete, involving only young-of-the-year fish, to complete. A marked selective mortality pattern was noted; smaller fish succumbed at lower dosages; as the concentrations were increased, larger fish were killed. Duration of toxicity did not appear excessive--five of the seven lakes in which mortality was complete were successfully restocked within 7 months after treatment.

Toxaphene (chlorated camphene) has not been widely used as a piscicide for local field application because minimum lethal concentrations in the field have not been determined. Considerable information has been gained from laboratory bioassay studies (Surber, 1948; Doudoroff et al., 1953; Hooper and Grzenda, 1957; Henderson et al., 1959), but the concentrations indicated by such studies are not necessarily correct for field use. Prevost (1960) pointed out that results of controlled laboratory experiments do not always yield dosages giving similar results in the field, where there are a number of variables, both known and unknown, over which the field worker has little or no control.

Gebhards (1960) in his review lists 14 Western States and 6 Canadian Provinces that have used toxaphene in fish-control programs in various formulations at concentrations ranging

from a low of 0.003 parts per million (p.p.m.) to a high of 0.61 p.p.m. Inconsistencies were emphasized in the review by the side variations in results. Fifteen of the reports said an average concentration of 0.185 p.p.m. failed to kill all fishes in treated areas, while 23 reports mention that an average concentration of 0.139 p.p.m. caused complete mortality. Stringer and McMynn (1958) reported complete kills at from 0.010 p.p.m. to 0.036 p.p.m.

In North Dakota, mortality of fish was complete when toxaphene was applied at a concentration of 0.070 p.p.m., which was not considered the minimum lethal level (Henegar, 1958). It was to determine the desirable minimum concentration for management use in North Dakota that this study was initiated.

Sixteen lakes were chosen for treatment during 1959 and 1960. All were test netted to determine existing populations of fish both before and after treatment. Physical and chemical characteristics were studied to establish criteria. Application of the toxaphene followed procedures commonly used by the North Dakota Game and Fish Department.

This publication is based on a thesis submitted to the Graduate Faculty, Department of Agriculture, South Dakota State College of Agriculture and Mechanic Arts, in partial fulfillment of the requirements for the degree of Master of Science, June 1961.

For lakes with physical and chemical characteristics of those in the Great Plains, recommended concentrations range from 0.025 to 0.030 p.p.m. as indicated in table 1. Concentrations used on the project areas ranged from 0.005 to 0.035 p.p.m.

TABLE 1.--Recommended concentration of toxaphene for various types of lakes.
[William Cooper & Nephews, Chicago, Ill., 1958]

Concentration of toxaphene	Lake type
25-30 p.p.b. ¹	Unstratified, shallow, hard-water lakes of low transparency and high productivity.
15-20 p.p.b.	Unstratified, soft-water lakes of moderate or high transparency.
10-20 p.p.b.	Stratified lakes of moderate depth (mean depth less than 20 feet) and moderate to low transparency.
7.5-10 p.p.b.	Stratified lakes of great depth (mean depth greater than 20 feet), high transparency, and low productivity.

¹ One report indicates that a concentration of 50-100 p.p.b. should be used in highly turbid waters containing suspended clay (Secchi disk reading less than 1 foot).

I wish to express my appreciation to Marvin O. Allum, Assistant Professor of Zoology, for his suggestions, interest, and constructive criticism during this study. I also wish to thank Dr. Donald Progulske, Assistant Professor of Zoology, for his advice and suggestions on preparation of the manuscript. I am obliged to all the members of the Fishery Division of the North Dakota Game and Fish Department who generously contributed time and suggestions on the field work.

CHARACTERISTICS OF TOXAPHENE

Toxaphene is one of the group of toxicants known as cyclodione insecticides, which also includes aldrin, dieldrin, chlordane, heptachlor, endrin, and isodrin. Toxaphene is not as well characterized chemically as the others, and the precise nature of the compounds in the mixture of isomers is not known (Negherbon, 1959). Most frequently encountered formulations are 10- to 20-percent dusts, 40-percent wettable powders, and emulsifiable concentrates of 4, 6, and 8 pounds per gallon.

The commercial product used in the study was Agricultural Cooper-Tox No. 6 (toxaphene emulsifiable concentrate) manufactured by

William Cooper & Nephews, Chicago, Ill. The formulation contained 6 pounds of technical toxaphene per gallon.

Toxaphene remains toxic for extended periods of time following application as a piscicide. Ten Michigan lakes treated in 1949 and 1950 detoxified in from 8 to 33 months. Other Michigan lakes detoxified in from 2 to 10 months after being treated at a concentration of 0.010 p.p.m. (Hooper and Grzenda, 1957). Stringer and McMynn (1960) reported that several British Columbia lakes remained toxic for over 2 years after treatment. Application at 1.00 p.p.m. (2.7 lbs. per acre-foot) gave concentrations of from 0.40 to 0.50 p.p.m. toxaphene.

Application rates do not appear to be important to the duration of toxicity when applied within the median tolerance limits of fishes. Mayhew (1959) stated that the period of toxicity is more related to chemical and physical characteristics of treated lakes than to the concentration of toxaphene.

The factors which influence detoxification are dilution, water temperature, water circulation, oxygen levels, turbidity, alkalinity, types of substrate, microorganisms, and ratio of water to bottom interface (Hemphill, 1954; Hooper and Grzenda, 1957; Rose, 1958; Henegar, 1958; Prevost, 1960).

Detoxification takes place very slowly when ice cover is present and more rapidly when water temperatures are high and conditions are favorable for the growth of plankton and other microorganisms.

METHOD OF APPLICATION

Toxaphene has been applied in various ways, the method usually depending on the formulation. The wettable powder has been applied by aircraft and by placing it in burlap bags and then towing in the wake of an outboard motor (Henegar, 1953). The emulsifiable liquid has been applied by aircraft, but most commonly by distributing premixed solutions with powder sprayers mounted in boats. One method is the metering of desired amounts into the wake of a

moving outboard-powered boat (Stringer and McMynn, 1960), but unless this is carefully controlled the toxaphene solution may settle to the bottom in a comparatively undiluted state.

In this study, a pumping system used by the North Dakota Game and Fish Department was employed (fig. 1). It consists of a 55-gallon drum connected to a motor-driven centrifugal pump with two outlet pipes terminating in common garden-type nozzles extending behind the boat transom on each side. During use, the nozzles are in contact with the surface of the lake to minimize airborne spray from coming in contact with the boat operator.

The toxaphene was premixed in the barrel at a maximum ratio of 1 gallon to 5 gallons of water so that a minimum of 55 gallons of liquid was available for spraying on each lake. During application the spray boat, powered by an 18-horsepower outboard motor, was operated at full throttle. This allowed for a maximum coverage of 200 acres an hour. On smaller lakes adjustment of the nozzles reduced application time to as little as 30 minutes.

The spray unit was left intact in the boat after each project, when the boat was loaded onto a boat trailer. This reduced the time necessary to ready equipment for each treatment.



Figure 1.--Spray boat used in application of toxaphene on project lakes.

Areas for treatment were determined from prepared hydrographic maps of each lake. Lakes over 100 acres in size were subsectioned, and each subsection was treated as a separate unit.

CHARACTERISTICS OF THE LAKES

For study I selected 16 lakes, varying in size from 6.3 to 915 acres, in widely scattered areas of North Dakota. Seven are impoundments, and nine are natural. Maximum depths ranged from 8 to 26 feet, and volume from 79 to 8,254 acre-feet (table 2). At the time of treatment, none of the lakes were chemically or thermally stratified.

Water samples were taken from all lakes, and field analyses were made to determine

chemical characteristics of the water (table 2). Concurrently, 1-gallon samples of water were forwarded to the North Dakota Health Department to check the accuracy of the field analyses. The variation in results between field and laboratory analyses was not significant.

With one exception, all the lakes had extensive growths of aquatic vegetation extending from shore outward to a depth of 8 feet. Potamogeton spp. and Myriophyllum spp. were particularly abundant, with Polygonum spp. and Sagittaria spp. prevailing less frequently. In all the lakes, primary bottom composition was silt, mud, clay, and organic material. Areas of sand, gravel, and rubble were of minor importance and were restricted to the natural lakes.

TABLE 2.--Physical and chemical characteristics of project lakes

Lake	Size	Maximum depth	Volume	pH	Alkalinity		Hardness	Total dissolved solids
					Phenolphthalein	Methyl orange		
	<u>Acres</u>	<u>Feet</u>	<u>Acre-feet</u>		<u>P.p.m.</u>	<u>P.p.m.</u>	<u>P.p.m.</u>	<u>P.p.m.</u>
Odland Lake.....	103.3	19	712.9	8.2	120	180	171	414
Brush Lake.....	160.2	23	1,526.7	8.5	40	460	476	290
Long Lake.....	290.8	23	2,390.6	8.3	40	220	308	307
Gums Lake.....	173.6	8	853.7	9.0	84	442	821	816
North Lake Metigoshe.....	670.8	22	7,588.5	8.8	20	210	205	317
South Lake Metigoshe.....	915.0	22	8,254.4	8.7	30	240	238	326
Red Willow Lake.....	129.9	26	1,272.8	8.0	24	178	239	364
Fretum Lake.....	95.2	22	511.7	8.3	54	496	444	281
North Lake Tobiasson.....	50.3	14	472.8	8.6	44	282	359	779
Bowbells Mine Lake.....	6.3	21	78.9	9.9	0	800	2,385	4,100
Glen Ullin Reservoir.....	12.3	18	144.4	8.0	60	420	136	418
South Lake Tobiasson.....	40.1	10	286.9	8.5	106	460	547	1,452
Nieuwsma Lake.....	80.8	23	651.2	7.9	0	140	136	415
Cat Coulee Lake.....	8.0	17	87.4	8.8	30	130	170	328
Wolf Butte Lake.....	17.3	14	137.5	9.4	100	260	114	940
Jund Lake.....	18.5	14	162.9	8.0	20	80	51	306

TEST NETTING

Qualitative sampling of fish populations in all lakes was carried out before and after treatment (table 3). Three types of gear were used, gill nets, small-mesh seines, and small-mesh frame nets.

The gill nets were experimental nylon nets, 250 by 6 feet, composed of 50-foot sections of increasing mesh size: 3/4, 1, 1-1/4, 1-1/2, and 2 inches. Total netting effort for each lake varied according to size; a greater number of sets were made in larger lakes. The nets were fished in diurnal and nocturnal periods to lend validity to the results. Although this type of gear is subject to considerable error, estimates of fish populations may be made from

the data. During the project period all lakes were gill-netted a total of 2,304 hours, representing 96 individual sets.

Shoreline seining with a 100- by 6-foot, 1/4-inch-mesh, nylon bag seine was done where vegetation was not too dense. Data so gathered were of limited value owing to

TABLE 3.--Fishes in project lakes.

Rainbow trout, Salmo gairdneri Richardson.
 Northern pike, Esox lucius Linnaeus.
 Carp, Cyprinus carpio Linnaeus.
 Golden shiner, Notemigonus crysoleucas (Mitchill).
 Bluntnose minnow, Pimephales notatus (Rafinesque).
 White sucker, Catostomus commersoni (Lacepede).
 Black bullhead, Ictalurus melas (Rafinesque).
 Brown bullhead, Ictalurus nebulosus (LeSueur).
 Orangespotted sunfish, Lepomis humilis (Girard).
 Bluegill, Lepomis macrochirus Rafinesque.
 White crappie, Pomoxis annularis Rafinesque.
 Black crappie, Pomoxis nigromaculatus (LeSueur).
 Yellow perch, Perca flavescens (Mitchill).
 Walleye, Stizostedion vitreum vitreum (Mitchill).

inconsistent results. Altogether, 71 drags were made in project lakes, representing a coverage of 35,500 square feet.

Small-mesh frame nets produced valid data on populations of fishes inhabiting the littoral zone. These nets were originally designed to sample young-of-the-year northern pike in heavily vegetated areas. The front frame of each net was 3 feet high and 4 feet wide (fig. 2); the net was 15 feet long and had round wooden hoops behind the front rectangular frame. The webbing was 1/4-inch nylon dyed a dark brown. The single tunnel used the string type of construction rather than open orifice, and the net retained the trapped fishes very satisfactorily. The single, 50-foot lead from the front frame was of the same material as the body of the net. In use, the frame nets were placed at right angles to the shoreline. A net of this type can be placed in position by one man, and sets are made without using a boat. Representative catches were made in all types of habitat. The frame nets were fished a total of 3,704 hours during the project period.



Figure 2.--Small-mesh frame net used to sample fishes in the littoral zone.

RESULTS

ODLAND LAKE

Odland Lake was treated on August 12, 1960, with toxaphene at a concentration of 0.005 p.p.m. No effects of the toxaphene were observed for 10 hours following application; after this period, distressed young-of-the-year yellow perch and black bullheads were noted in the shallow bays and backwaters. Within 36 hours many of these small fishes were either lying dead on the bottom or washed ashore. Evident mortality ceased 48 hours after application. In the observation period only seven larger fish were noticeably affected. The small fish decomposed rapidly, and after 7 days evidence of their mortality disappeared.

The lake was test-netted 48 days after treatment. Net frequencies (table 5) were not significantly changed from those arrived at before the toxaphene was used. In this lake the net frequencies did not show the true population

TABLE 4.--Concentrations of toxaphene applied and fish mortality in project lakes

Lake	Concentration (p.p.m.)	Mortality
Odland Lake.....	0.005	Incomplete
Brush Lake.....	0.010	Incomplete
Long Lake.....	0.010	Incomplete
Gumms Lake.....	0.010	Incomplete
North Lake Metigoshe.....	0.015	Incomplete
South Lake Metigoshe.....	0.015	Incomplete
Red Willow Lake.....	0.015	Incomplete
Fretum Lake.....	0.020	Incomplete
North Lake Tobiason.....	0.020	Incomplete
Bowbells Mine Lake.....	0.025	Complete
Glen Ullin Reservoir.....	0.025	Complete
South Lake Tobiason.....	0.025	Complete
Nieuwsma Lake.....	0.025	Complete
Cat Coulee Lake.....	0.030	Complete
Wolf Butte Lake.....	0.030	Complete
Jund Lake.....	0.035	Complete

TABLE 5.--Changes in test-netting frequencies following application of toxaphene in project lakes

[Frequencies are computed as fish per hour per net both for frame and gill nets. Seining data not used]

	Concentration (p.p.m.)	Frequency		Reduction (Percent)
		Before treatment	After treatment	
Odland Lake.....	0.005	6.91	6.94	None
Brush Lake.....	0.010	6.88	1.34	80.5
Long Lake.....	0.010	10.38	1.59	84.6
Gumms Lake.....	0.010	34.46	7.96	76.9
North Lake Metigoshe.....	0.015	8.46	1.07	87.3
South Lake Metigoshe.....	0.015	6.46	1.11	83.2
Red Willow Lake.....	0.015	10.20	2.50	75.4
Fretum Lake.....	0.020	6.36	.32	94.9
North Tobiason Lake.....	0.020	12.91	.33	97.4
Bowbells Mine Lake.....	0.025	.95	0.00	100.0
Glen Ullin Reservoir.....	0.025	6.91	0.00	100.0
South Lake Tobiason.....	0.025	7.29	0.00	100.0
Nieuwsma Lake.....	0.025	5.89	0.00	100.0
Cat Coulee Lake.....	0.030	4.06	0.00	100.0
Wolf Butte Lake.....	0.030	15.08	0.00	100.0
Jund Lake.....	0.035	3.62	0.00	100.0

structure. Young-of-the-year fishes taken during pretreatment test netting (table 6) were absent from the data. Average sizes of larger fishes remained relatively stable, reflecting insignificant mortality.

BRUSH LAKE

Brush Lake was treated on October 5, 1959, with toxaphene at a concentration of 0.010 p.p.m. Application preceded lake freeze-up by only 2 days. During the 2 days that observations could be made, no affected fish were found. The following spring (157 days after treatment) thousands of partly decomposed yellow perch (2.1 to 6.1 inches) were washed on shore. Seventeen walleyes (9.8 to 12.3 inches) and five northern pike (10.1 to 13.1 inches) were recorded. Further observations for 5 days after breakup did not reveal additional current mortality.

Ninety-six hours of posttreatment test netting starting on April 22, 1960, disclosed only a partial mortality among the fishes. It was evident from the results (table 7) that although mortality was heavy among the young-of-the-year and older yellow perch (2.1 to 5.9 inches), little difference in the abundance of the larger fishes could be found. Total test netting frequency was reduced by 80.5 percent (table 5).

On May 27, 1960, Brush Lake was stocked with 29,000 northern pike fingerlings (2,000/lb.). On June 26, 1960, it was stocked with 48,000 walleye fingerlings (1,600/lb.) and on August 1, 1960, with 200,000 bluntnose minnows (1,000/lb.).

TABLE 6.--Odland Lake test netting data before and after treatment with 0.005 p.p.m. toxaphene

[Combined data from all types of netting gear]

Species	Number	Length (inches)	
		Range	Average
Before treatment (134 hours):			
Black bullhead.....	418	4.0 - 11.6	5.8
Do.....	405	young-of-year	--
Yellow perch.....	44	3.4 - 6.5	5.5
Golden shiner.....	27	5.1 - 6.5	6.1
Northern pike.....	17	16.0 - 28.3	21.7
White sucker.....	9	9.0 - 18.5	13.6
White crappie.....	3	7.5 - 8.5	8.0
Orangespotted sunfish.....	2	5.5 - 5.6	5.5
After treatment (152 hours):			
Black bullhead.....	904	5.0 - 10.5	5.7
Do.....	0	young-of-year	--
Northern pike.....	59	15.0 - 29.0	21.8
Yellow perch.....	48	3.5 - 12.5	5.8
White crappie.....	19	5.5 - 9.0	7.3
Golden shiner.....	17	6.0 - 7.1	6.5
Orangespotted sunfish.....	11	5.5 - 6.0	5.6
White sucker.....	1	16.7	17.7

TABLE 7.--Brush Lake test netting data before and after treatment with 0.010 p.p.m. toxaphene

[Combined data from all types of netting gear]

Species	Number	Length (inches)	
		Range	Average
Before treatment (144 hours):			
Yellow perch.....	908	2.6 - 13.7	4.1
Do.....	894	young-of-year	--
Walleye.....	49	9.7 - 17.7	15.6
Northern pike.....	32	10.3 - 27.2	26.3
White sucker.....	4	10.1 - 10.5	10.3
After treatment (96 hours):			
Yellow perch.....	57	6.0 - 12.8	7.2
Do.....	0	young-of-year	--
Northern pike.....	56	17.6 - 30.2	22.4
Walleye.....	39	15.8 - 18.1	16.1
White sucker.....	7	10.8 - 11.1	10.9

Additional test netting of the lake in September proved good survival and growth of all stocked fishes. This test netting also substantiated results from the posttreatment survey.

LONG LAKE

Long Lake was treated on July 17, 1960, with toxaphene at a concentration of 0.010 p.p.m. Mortality during the first 3 hours after application was light and was restricted to young-of-the-year yellow perch. At the end of 5 hours, yearling and 2-year-old yellow perch were in distress. Twenty hours later yellow perch up to 5.1 inches were either moribund or dead in all sections of the lake. At this time distressed and dying young-of-the-year northern pike were found. Deaths continued until 72 hours after treatment, when the last affected fish were observed. During the apparent period of toxicity only two dead 10-inch walleyes and no northern pike larger than young-of-the-year were observed. No dead white suckers could be located.

On August 18, approximately 1 month after treatment, Long Lake was stocked with 160,000 (360/lb.) bluntnose minnows. Periodic observations for 10 days after stocking indicated no mortality of these fishes.

During posttreatment test netting begun on October 25, the stocked minnows were frequently taken in the small-mesh frame nets. Yields of these small-mesh frame nets (table 8) denoted that the toxaphene had either severely reduced or eliminated yellow perch less than 5.5 inches in length. Partial reduction of young-of-the-year northern pike was evident.

TABLE 8.--Long Lake test netting before and after treatment with 0.010 p.p.m. toxaphene

[Combined data from all types of netting gear]

Species	Number	Length (inches)	
		Range	Average
Before treatment (250 hours):			
Yellow perch.....	1,240	2.6 - 6.8	3.8
Do.....	1,017	young-of-year	--
Northern pike.....	6	26.0 - 31.3	28.2
Do.....	327	young-of-year	--
Walleye.....	3	10.0 - 10.3	10.1
After treatment (250 hours):			
Yellow perch.....	263	5.6 - 6.8	6.5
Do.....	0	young-of-year	--
Northern pike.....	5	25.1 - 32.1	28.6
Do.....	114	young-of-year	--
Walleye.....	3	10.3 - 10.6	10.4
White sucker.....	3	17.3 - 17.6	17.5

Total net frequency on all fishes was reduced by 84.6 percent (table 5). No measurable changes were found in the populations of adult northern pike, walleyes, or white suckers (table 8).

GUMMS LAKE

Gumms Lake was treated on August 8, 1959, with toxaphene at a concentration of 0.015 p.p.m. At time of treatment the lake contained the largest yellow perch population of all project lakes (table 9). One hour after application, small affected yellow perch were seen over most of the surface of the lake. After 3 hours many yellow perch (2.4 to 6.6 inches) were either distressed or dead. This condition was maintained for 26 hours, after which the incidence started a rapid decline. The last moribund fish was located 71 hours after treatment, and by this time thousands of yellow perch lined the shore and were floating on the lake. During the period of mortality no yellow perch larger than 7 inches were seen.

Test netting on September 14, 1958, revealed an incomplete kill. No yellow perch smaller than 5.5 inches were taken, but the abundance of larger yellow perch remained unchanged (table 9). Average size of fishes taken during this posttreatment netting increased from 5.3 to 7.8 inches (table 9). Total net frequency indicated a gross reduction of 87.3 percent (table 5).

Plans for additional test netting of this lake in 1960 were abandoned after winter-kill conditions during the winter of 1959-60.

TABLE 9.--Gumms Lake test netting before and after treatment with 0.010 p.p.m. toxaphene

[Combined data from all types of netting gear]

Species	Number	Length (inches)	
		Range	Average
Before treatment (28 hours):			
Yellow perch.....	964	3.1 - 10.2	5.3
After treatment (28 hours):			
Yellow perch.....	223	7.0 - 10.3	7.8

NORTH LAKE METIGOSHE

North Lake Metigoshe was treated on September 13, 1960, with toxaphene at a concentration of 0.015 p.p.m. It was the second largest lake in the project and contained the most desirable fish population. Because of its size, it was treated in segments, and each was sprayed as a separate unit. Application was completed in 6 hours.

Reaction of the fishes to the toxaphene was slow because of low water temperature of 43 F., but by the end of 14 hours following completion of treatment surfacing of small fishes was noted. Young-of-the-year yellow perch and black bullheads were affected first, followed by larger perch (2.0 to 3.2 inches) and bullheads (2.2 to 5.8 inches) after 20 hours. Thirty-six hours later, numbers of dead and affected perch and bullheads were blown to the north shore by a strong southerly wind that prevailed for 10 hours. Frequency of distressed fishes was highest after 72 hours, after which there was a rapid decline. All activity had ceased at the end of 12 days.

Observations of gross mortality after 96 hours disclosed countless yellow perch ranging from young-of-the-year to 5.1 inches in length, 181 northern pike (11.9 to 18.4 inches), and 17 walleyes (14.2 to 15.2 inches). No mortality of white suckers was noted. Observers checking the bays and shallow water failed to find any living bullheads or yellow perch.

Posttreatment netting was delayed to 3 days before freeze-up (October 24). Observations made on the lake during the period of toxicity were confirmed by the results of the test netting (table 10). The toxaphene had failed to kill all fishes in the lake (fig. 3). Yellow perch

TABLE 10.--North Lake Metigoshe test netting data before and after treatment with 0.015 p.p.m. toxaphene

[Combined data from all types of netting gear]

Species	Number	Length (inches)	
		Range	Average
Before treatment (322 hours):			
Yellow perch.....	1,305	2.4 - 10.1	4.2
Do.....	626	young-of-year	--
Black bullhead.....	416	2.1 - 11.1	4.8
Do.....	1,438	young-of-year	--
Walleye.....	46	13.0 - 25.3	17.6
Northern pike.....	27	12.0 - 24.8	16.1
Bluntnose minnow.....	17	--	--
White sucker.....	9	17.7 - 23.0	19.9
After treatment (322 hours):			
Yellow perch.....	49	4.3 - 9.6	6.8
Do.....	0	young-of-year	--
Black bullhead.....	246	6.1 - 10.2	7.6
Do.....	0	young-of-year	--
Walleye.....	39	19.1 - 26.2	22.8
Northern pike.....	15	13.3 - 24.7	19.3
White sucker.....	8	17.6 - 24.0	19.8



Figure 3.--Fish taken in gill net from North Lake Metigoshe after treatment with 0.015 p.p.m. toxaphene.

from young-of-the-year to 4.0 inches and black bullheads from young-of-the-year to 5.8 inches were absent from the nets. The average sizes of yellow perch and black bullheads increased from 4.1 inches to 6.8 inches and 4.8 inches to 7.6 inches respectively (table 10). Average sizes of northern pike, walleyes, and white

suckers stayed approximately the same. Reduction in total test net frequency was 87.3 percent (table 5).

SOUTH LAKE METIGOSHE

South Lake Metigoshe was treated on July 7, 1960, with toxaphene at a concentration of 0.015 p.p.m. It was the largest lake in the project and for convenience was divided into segments for treatment. Application of the toxaphene was completed in 7 hours.

High water temperature (75° F.) at time of treatment caused the toxaphene to act rapidly on the fishes. One hour after starting application small yellow perch and black bullheads were surfacing. Frequency of distress increased rapidly, and at the end of 2 hours moribund and dead fishes were seen in treated areas (fig. 4). Rate of death increased in all parts of the lake for another 48 hours and then began a rapid decrease. Ninety-six hours later



Figure 4.--Moribund and dead young-of-the-year yellow perch taken at South Lake Metigoshe 4 hours after treatment with 0.015 p.p.m. of toxaphene.

major activity had stopped. Ten days later a discontinuation of activity was obvious.

Five to 7 days after treatment, "float-up" took place as thousands of decomposing fish floated into shore. This caused a public-relations problem of some magnitude with the 491 cabin owners on the lake and emphasized the value of fall treatment. Mortality estimates made at the time were of questionable value because fishes were picked up and disposed of as soon as they came to shore. It seemed, however, that while many smaller fishes had succumbed, the larger fishes were unaffected.

Posttreatment test netting (table 11) from September 17 to 20 proved the kill was incomplete. Young-of-the-year yellow perch, black bullheads, and northern pike were severely reduced. The only area containing any of these fishes was close to the entrance from North Lake Metigoshe. It is probable that they migrated into the area from this entrance following detoxification.

Yellow perch (2.4 to 4.0 inches) and black bullheads (2.1 to 4.2 inches) were absent from the population samples taken after treatment. Frequencies and average sizes of the northern pike, walleyes, and white suckers in the post-treatment population were relatively the same as before treatment. Total test netting frequency was reduced by 83.2 percent (table 5).

After the presence of walleyes and northern pike was established by the posttreatment net-

ting, some sport fishing took place. A small number of both fishes were taken until fishing was halted by freeze-up.

RED WILLOW LAKE

Red Willow Lake was treated on July 17, 1959, with toxaphene at a concentration of 0.020 p.p.m. During application the water temperature was 72° F., and action of the toxicant was rapid. Small yellow perch and black bullheads surfaced 1 hour after spraying was begun. Surfacing movements became more rapid and then waned at the end of 72 hours. Many yellow perch (3.1 to 6.8 inches) and black bullheads (3.2 to 5.6 inches) were discovered in the vegetation and along the shore. Observations also disclosed 73 dead northern pike (12.4 to 17.6 inches) and only 2 dead white suckers (17.2 inches).

Test netting of the lake on October 13, 1959, (table 12) disclosed an incomplete kill of the fish population. The reduction of yellow perch was of consequence, but the black bullheads retained their original abundance. Average length of the yellow perch increased from 5.3 to 6.9 inches, while the average length of the black bullheads increased only from 6.8 to 6.9 inches. Frequencies of larger fishes failed to display a positive reduction. Total net frequency was lowered by 75.4 percent (table 5).

FRETTUM LAKE

Frettum Lake was treated on July 19, 1959, with toxaphene at a concentration of 0.020 p.p.m. Pretreatment test netting of this lake

TABLE 11.--South Lake Metigoshe test netting data before and after treatment with 0.015 p.p.m. toxaphene

[Combined data from all types of netting gear]

Species	Number	Length (inches)	
		Range	Average
Before treatment (333 hours):			
Yellow perch.....	2,984	2.4 - 9.5	4.0
Do.....	2,024	young-of-year	--
Black bullhead.....	737	2.1 - 9.7	6.7
Do.....	814	young-of-year	--
Northern pike.....	18	10.7 - 21.7	18.1
Do.....	142	young-of-year	6.1
Walleye.....	19	7.5 - 25.5	18.3
Bluntnose minnow.....	2	--	--
After treatment (290 hours):			
Yellow perch.....	104	4.1 - 9.4	9.3
Do.....	17	young-of-year	--
Black bullhead.....	520	4.2 - 9.6	7.9
Do.....	26	young-of-year	--
Northern pike.....	19	14.1 - 22.3	20.1
Do.....	28	young-of-year	9.1
Walleye.....	22	12.1 - 24.2	18.6

TABLE 12.--Red Willow Lake test netting data before and after treatment with 0.015 p.p.m. toxaphene

[Combined data from all types of netting gear]

Species	Number	Length (inches)	
		Range	Average
Before treatment (48 hours):			
Yellow perch.....	351	3.7 - 7.1	5.3
Black bullhead.....	120	3.8 - 9.0	6.8
Northern pike.....	12	13.0 - 27.5	19.9
White sucker.....	7	14.0 - 18.5	17.4
After treatment (48 hours):			
Yellow perch.....	5	6.6 - 7.2	6.9
Black bullhead.....	103	3.9 - 8.2	6.9
Northern pike.....	13	9.0 - 16.7	14.7
White sucker.....	2	14.9 - 15.0	14.9

revealed a dense population of yellow perch except for young-of-the-year. In only 3 hours after application, numerous fishes were either dead or distressed. After 96 hours further movement could not be found. This initial high mortality of the perch suggested the possibility of a complete kill. Posttreatment test netting on October 21, 1959, proved this assumption to be incorrect. Although smaller yellow perch (4.0 to 6.4 inches) were absent from the test nets, larger yellow perch were taken (table 13). The average length of the yellow perch increased from 5.3 to 7.3 inches, and percent reduction of total netting frequency was 94.9 (table 5).

TABLE 13.--Fretum Lake test netting data before and after treatment with 0.020 p.p.m. toxaphene

[Combined data from all types of netting gear]

Species	Number	Length (inches)	
		Range	Average
Before treatment (120 hours):			
Yellow perch.....	429	4.1 - 14.0	5.3
Do.....	335	young-of-year	--
After treatment (116 hours):			
Yellow perch.....	38	6.5 - 14.1	7.3
Do.....	0	young-of-year	--

NORTH LAKE TOBIASON

North Lake Tobiason was treated on August 6, 1959, with toxaphene at a concentration of 0.020 p.p.m. Reaction of fishes to the toxaphene began 1 hour after spraying was started, and after 4 hours yellow perch and brown bullheads were surfacing over the entire lake. This activity gained in intensity for another 68 hours and then dwindled to nothing after 72 hours. When apparent toxicity ceased, examination of the dead fish revealed many dead yellow perch and brown bullheads but only five dead northern pike (19.2 to 20.5 inches).

This lake was not test netted until 10 months after treatment (June 7, 1960), and results ascertained that only a partial kill had taken place. Brown bullheads were absent from the test nets. No yellow perch were taken in the samples, and the only fishes surviving were larger northern pike and white suckers (table 14). The percent reduction in total net frequency was 97.4 (table 5).

TABLE 14.--North Lake Tobiason test netting data before and after treatment with 0.020 p.p.m. toxaphene

[Combined data from all types of netting gear]

Species	Number	Length (inches)	
		Range	Average
Before treatment (48 hours):			
Yellow perch.....	446	3.1 - 7.2	5.3
Brown bullhead.....	160	4.2 - 10.2	7.8
Northern pike.....	5	19.1 - 26.2	23.7
White sucker.....	6	16.4 - 19.0	17.2
White crappie.....	4	4.3 - 4.5	4.4
After treatment (48 hours):			
Yellow perch.....	0	--	--
White sucker.....	8	16.5 - 19.0	17.3
Northern pike.....	7	21.0 - 26.1	24.2

BOWBELLS MINE LAKE

Bowbells Mine Lake was treated on August 28, 1959, with toxaphene at a concentration of 0.025 p.p.m. The lake, which was the smallest of the series involved, manifested extremes in water chemistry (table 2). The origin of the lake is seepage of ground water into an abandoned lignite-coal strip mine. The toxicant acted more slowly on the fishes than in any other project lake, and 6 hours elapsed before the first distressed yellow perch were visible. The frequency of kill increased gradually for 96 hours after which it dropped off rapidly, and at the end of 10 days a cessation of activity was noted.

Posttreatment test netting (table 15) was not carried out until May 9, 1960, at which time no fish were taken. The lake was then restocked on September 21, 1960, with 2,200 rainbow trout (2.8/lb.). Test netting in November 1960 disclosed good survival and additional growth of the trout.

GLEN ULLIN RESERVOIR

Glen Ullin Reservoir was treated on July 23, 1959, with toxaphene at a concentration of 0.025 p.p.m. Fishes reacted rapidly to the toxicant, and within 4 hours after spraying was begun distressed fish were in evidence in all areas of the lake. After 17 hours, mortality of white crappie and yellow perch appeared complete. No carp or white suckers were observed until 14 hours after treatment, but all had apparently died after 35 hours. Continued observations for 7 days disclosed no further mortality. Two days of posttreatment test netting on September 26, 1959, and May 17, 1960, did not yield any live fish (table 16). The

TABLE 15.--Bowbells Mine Lake test netting data before and after treatment with 0.025 p.p.m. toxaphene

[Combined data from all types of netting gear]

Species	Number	Length (inches)	
		Range	Average
Before treatment (24 hours):			
Yellow perch.....	17	5.1 - 8.3	7.2
White sucker.....	6	12.2 - 17.1	14.2
After treatment (48 hours):			
No fish taken.....	--	--	--

TABLE 16.--Glen Ullin Reservoir test netting data before and after treatment with 0.025 p.p.m. toxaphene

[Combined data from all types of netting gear]

Species	Number	Length (inches)	
		Range	Average
Before treatment (24 hours):			
White crappie.....	160	3.1 - 5.0	4.5
Yellow perch.....	3	4.7 - 5.2	5.0
Carp.....	2	13.1 - 13.4	13.25
White sucker.....	1	13.0	13.0
After treatment (48 hours):			
No fish taken.....	--	--	--

lake was then restocked with 5,000 fingerling largemouth bass, 572 adult white crappies, 90,000 bluntnose minnows, and 7,500 fingerling bluegills. Subsequent test netting in November 1960 indicated good survival and growth of these fishes.

SOUTH LAKE TOBIASON

South Lake Tobiason was treated on August 7, 1959, with toxaphene at a concentration of 0.025 p.p.m. Six hours after treatment, large numbers of distressed or dead black bullheads were found around the shore or floating on the surface. Forty-eight hours later no further mortality was apparent.

Posttreatment test netting on October 9, 1959, and April 12, 1960, failed to take any live fishes (table 17).

NIEUWSMA LAKE

Nieuwsma Lake had been treated in 1957 with emulsifiable rotenone at a concentration of 1.00 p.p.m. The treatment was unsuccessful in killing all of the black bullheads. Plantings of northern pike and bluegills in 1958 were of limited value. Retreatment of the lake with a concentration of 0.025 p.p.m. of toxaphene was

TABLE 17.--South Lake Tobiason test netting before and after treatment with 0.025 p.p.m. toxaphene

[Combined data from all types of netting gear]

Species	Number	Length (inches)	
		Range	Average
Before treatment (24 hours):			
Black bullhead.....	172	5.5 - 6.9	6.2
Northern pike.....	2	12.0	12.0
Yellow perch.....	1	6.2	6.2
After treatment (96 hours):			
No fish taken.....	--	--	--

carried out on June 7, 1960. Five hours after beginning the application, large numbers of black bullheads could be seen at the surface. Mortality continued for 120 hours and then dwindled until after 145 hours no further movement was observed.

On October 2, 1960, posttreatment test netting failed to produce any live fish from the lake (table 18).

CAT COULEE LAKE

Cat Coulee Lake had been previously treated twice (1.00 p.p.m. and 2.00 p.p.m. of emulsifiable rotenone) to eradicate a population of black bullheads. Both applications were unsuccessful. On July 15, 1959, the lake was re-treated with toxaphene at a concentration of 0.030 p.p.m. Within 5 hours of starting the application, distressed black bullheads were to be found in all areas of the lake. The few rainbow trout in the lake succumbed quickly, and the last moribund bullhead was observed 96 hours later.

No fish were caught in test netting (table 19) on May 4, 1960. The lake was restocked on September 23, 1960, with 4,600 rainbow trout (600/lb.).

WOLF BUTTE LAKE

In 1957 Wolf Butte Lake was treated with 1.00 p.p.m. of emulsifiable rotenone to remove a population of bullheads and green sunfish. The application was unsuccessful. Rainbow trout that had been stocked into the area after treatment furnished angling for one season, and

TABLE 18.--Nieuwsma Lake test netting before and after treatment with 0.025 p.p.m. toxaphene

[Combined data from all types of netting gear]

Species	Number	Length (inches)	
		Range	Average
Before treatment (48 hours):			
Black bullhead.....	771	2.3 - 7.1	5.5
Northern pike.....	12	11.1 - 16.3	14.8
After treatment (72 hours):			
No fish taken.....	--	--	--

TABLE 19.--Cat Coulee Lake test netting data before and after treatment with 0.030 p.p.m. toxaphene

[Combined data from all types of netting gear]

Species	Number	Length (inches)	
		Range	Average
Before treatment (48 hours):			
Black bullhead.....	194	3.1 - 6.2	4.1
Rainbow trout.....	5	9.0 - 12.3	9.7
After treatment (48 hours):			
No fish taken.....	--	--	--

the green sunfish and bullheads quickly regained their original abundance. After this the trout fishing began a rapid decline.

The lake was retreated on July 9, 1960, with toxaphene at a concentration of 0.030 p.p.m. The fish reacted slowly to the toxaphene, and it was not until 21 hours after treatment that any activity was noted. Surfacing of distressed and dying fishes continued for 7 days, and then no further activity was observed.

Posttreatment test netting carried out on September 8, 1960, indicated all fish had succumbed (table 20).

JUND LAKE

Jund Lake was treated on June 9, 1960, with toxaphene at a concentration of 0.035 p.p.m. Action of the toxicant was rapid, and within 2 hours many dead small bullheads were in evidence. Activity increased for 24 hours and then declined at a rapid rate. After 72 hours no additional mortality was found.

On October 5, 1960, the lake was test netted, and negative results indicated complete mortality (table 21).

TABLE 20.--Wolf Butte Lake test netting data before and after treatment with 0.030 p.p.m. toxaphene

[Combined data from all types of netting gear]

Species	Number	Length (inches)	
		Range	Average
Before treatment (72 hours):			
Green sunfish.....	1,003	1.8 - 5.3	4.0
Black bullhead.....	68	4.2 - 8.1	7.1
Rainbow trout.....	18	10.2 - 15.2	13.1
After treatment (72 hours):			
No fish taken.....	--	--	--

TABLE 21.--Jund Lake test netting data before and after treatment with 0.035 p.p.m. toxaphene

[Combined data from all types of netting gear]

Species	Number	Length (inches)	
		Range	Average
Before treatment (48 hours):			
Black bullhead.....	163	3.1 - 5.9	4.8
Rainbow trout.....	1	15.7	15.7
After treatment (48 hours):			
No fish taken.....	--	--	--

DISCUSSION AND CONCLUSIONS

The mortality pattern following application of various concentrations of toxaphene was consistent, and a marked size selectivity was exhibited. As concentration rates were increased, the extent of mortality of larger fishes increased until the minimum lethal level (when all fishes succumbed) was reached. From results on the 16 project lakes it appears that the minimum lethal concentration for treatment of most North Dakota lakes is 0.025 p.p.m. of toxaphene. Concentrations progressively less than this induce mortality only on progressively smaller fishes.

The use of sublethal dosages of toxaphene for partial population removal is indicated. In all lakes treated below lethal concentrations, smaller fishes were removed leaving the larger fishes relatively unharmed. In lakes without rough fish populations this removal of small, undesirable fishes could have beneficial effects on the resulting fishery. The need for further research along this line is pointed out.

Present methods of applying toxaphene are satisfactory, for during the project period no difficulty was experienced. Observed mortality patterns indicate advantages to spending more

than the minimum time necessary for application. This would be of greater importance if toxaphene were being used for partial population removal. More thorough distribution of the toxaphene would give more homogeneous immediate concentration, negating the problem of mortality among the desirable larger fishes from high initial unmixed concentrations. It is also conceivable that in larger lakes (500 acres or more), under conditions of rapid detoxification, the concentration of toxaphene could be lowered to less than the minimum lethal level before complete mixing.

No marked correlation between water chemistry and rate of reaction of fishes to toxaphene was noted. It did appear that in highly alkaline waters the toxicant acted somewhat slower, but inasmuch as all lakes were alkaline in nature this was not definite. Temperature was the factor determining the rate of reaction; the lower the temperature of the water the slower the reaction of the fishes to the toxaphene. Results from Brush Lake prove that toxaphene will detoxify even while the lake is under ice cover.

Actual detoxification rates were not studied during the project period. It must be assumed that in lakes where kills were incomplete the initial concentration was below minimum lethal level or detoxification at these low dosages is more rapid than heretofore believed.

Five of the seven lakes in which mortality was complete were restocked within 7 months of treatment, and good survival and growth has been noted among the stocked fishes.

In all lakes, increase in transparency was evident following application of the toxaphene. This could have been related to the removal of fishes that kept materials in suspension. In some cases, however, it was more probable that the increase in transparency was due to limited flocculation caused by the toxaphene or its solvents.

No ill effects were felt by personnel working with the toxaphene. It appears that if toxaphene is judiciously handled, there is little danger in its use. It was found advantageous, however, to direct the spray downward to reduce airborne spray which is irritating to the eyes.

SUMMARY

During the summers of 1959 and 1960, 16 North Dakota lakes ranging from 6.3 to 915 acres were treated with toxaphene to remove populations of fishes. Objective of the study was to determine the minimum lethal concentration of toxaphene necessary for lake eradication projects.

Concentrations of toxaphene used on the lakes varied from 0.005 p.p.m. to 0.035 p.p.m. Incomplete mortality of fishes resulted in lakes at concentrations less than 0.025 p.p.m. At concentrations of 0.025 p.p.m. to 0.035 p.p.m. complete mortality was indicated, subject to the validity of test netting results.

Duration of toxicity of the toxaphene was not excessive. Five of the seven lakes in which kills were complete were successfully restocked within 7 months after treatment.

Results indicate that the North Dakota Game and Fish Department can use toxaphene at concentrations of 0.025 p.p.m. to 0.035 p.p.m. with reasonable assurance of killing all fishes in the treated lakes.

All lakes displayed a definite mortality pattern; the small fishes were the first to die, and the largest fishes were last. At the lowest project dosage of 0.005 p.p.m. only young-of-the-year were affected. The use of toxaphene as a size selective piscicide is strongly suggested.

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INVESTIGATIONS IN FISH CONTROL

**4. Effects of Toxaphene on
Plankton and Aquatic Invertebrates
in North Dakota Lakes**

By Robert G. Needham, Fishery Biologist
Montana Department of Fish and Game



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EFFECTS OF TOXAPHENE ON PLANKTON AND AQUATIC INVERTEBRATES IN NORTH DAKOTA LAKES

By Robert G. Needham, Fishery Biologist
Montana Department of Fish and Game

Abstract.--Effects of low concentrations of toxaphene on plankton and larger invertebrates were studied in four North Dakota lakes (a fifth lake, untreated, was a control). Brachionus, Keratella, Trichocerca, Asplanchna, Polyarthra, Conochiloides, Daphnia, Ceriodaphnia, Bosmina, and Cyclops were dominant zooplankters; none exhibited marked reduction after treatment at 5 to 34 p.p.b. Most phytoplankter populations showed no obvious changes after treatment, except Aphanizomenon, which increased in all lakes. The posttreatment increase in South Lake Metigoshe was especially noticeable, since there was no increase in untreated North Lake Metigoshe. Several of the plant-inhabiting and bottom fauna decreased slightly after treatment, but this did not appear to be the result of toxaphene treatment. Tolerance levels for several zooplankters and other aquatic invertebrates were observed in controlled experiments. Rotifera was the most tolerant group, followed in order by Cladocera and Copepoda. Among larger invertebrates, Hirudinea, Hydracarina, and Gastropoda were the most tolerant, followed in order by Trichoptera, Odonata, Hemiptera, Ephemeroptera, Amphipoda, and Coleoptera.

Use of toxicants in fishery management has provided considerable information concerning the effects of various poisons on fish. Much less is known of the effects on the fish-food organisms--several workers have reported some such effects: Hooper and Grzenda, 1957, in Michigan; Hoffman and Olive, 1961, and Cushing and Olive, 1957, in Colorado; and Stringer and McMynn, 1958, in British Columbia.

The object of this study was to determine the effects of toxaphene at low concentrations on the plankton and certain other aquatic organisms under natural and controlled conditions. This was made possible by the rough-fish removal program in North Dakota, which various concentrations of toxaphene were used. Investi-

gations were carried out in four lakes--a natural lake in the north-central part of the State and three impoundments in the southwest. A fifth lake, untreated, was a control. The study extended from June through September of both 1960 and 1961.

Dr. C. J. D. Brown directed the study; Dale L. Henegar, Chief of Fisheries, North Dakota Game and Fish Department, suggested the problem; I am indebted also to Dr. John C. Wright and Dr. G. W. Prescott for help in identifying plankton, to Dr. George F. Edmunds, Jr., for help in identifying aquatic insects, to Donald C. Warnick for help in field work, and to my wife Avis for help in analysing samples. Chemical analyses were made by the State Laboratories. The fish studies were by the North Dakota Fish and Game Department, which also provided financial aid under Dingell-Johnson Projects F-2-R 7 and 9. The National Wildlife Federation granted a fellowship for the last year of the study.

This publication is based on a thesis submitted to the Graduate Faculty, Montana State College, in partial fulfillment of the requirements for the degree of Master of Science in Fish and Wildlife Management, March 1962.

METHODS

Surface water temperatures were obtained with a pocket thermometer, and depth temperatures with a reversing thermometer. Secchi disk readings were taken at all stations in conjunction with each collection series.

The toxaphene used was an emulsified concentrate containing 6 pounds of technical toxaphene per gallon. Before application the toxaphene was diluted to 10 to 15 times with water to facilitate uniform distribution. It was applied to the water surface by spraying from a boat.

Water samples were collected before and after toxaphene treatment in 1960, and once in 1961. A summary of the physical and chemical data is presented in table 1.

Plankton samples were secured with a pump at 1.5 and 7.5 feet at all stations. All samples were taken while the boat was moving in order to avoid resampling the same water. Each sample contained 40 gallons of water, and two samples constituted a collection. Each sample was concentrated to 200 cc. with a No. 20 silk plankton net. Plankton counts consisted in total enumeration of all organisms in 1 cc., with the exception of a few abundant phytoplankters, which were counted by the differential method, employing 20 to 80 fields within a 1-cc. sample.

Plant-inhabiting organisms were collected with a metal device that I designed. This had an opening of 1 square foot and a height of 30 inches. Openings (4 by 6 inches) were cut on two sides to allow for drainage; these were covered by screen having 30 meshes per inch. A sliding plate was installed at the bottom to sever the plants near their roots. Samples were limited to water depths of 2 feet or less, since this device had to be operated manually. Approximately 4.5 pounds (drained weight, 2-3 minutes) of plants were taken per sample in 1960. In 1961 this was reduced to approximately 12 ounces, since analyses showed this to be adequate. The number of square feet of bottom covered in each sample varied from 8 to 16 in 1960 and from 3 to 5 in 1961.

Bottom organisms were taken with an Ekman dredge at depths ranging from 4 to 10 feet. Either 3 or 4 square feet were sampled at each station. Organisms from both plant and bottom samples were concentrated with a screen having 30 meshes per inch.

Plant-inhabiting organisms and bottom fauna were sampled at the same stations, which were approximately 50 feet in diameter. These stations had both abundant vegetation and open water.

TABLE 1.--Physical and chemical data before and after toxaphene treatment for two lakes and three reservoirs in North Dakota.

[All chemical data except pH are expressed as parts per million; bottom temperatures were taken at depths of 9-12 feet]

Sampling dates	Temperature (°F)		Secchi disk (feet)	Total solids	Total hardness	pH	Total alkalinity	Chlorides	Sulfates	Iron
	Surface	Bottom								
Wolfe Butte Reservoir (treated Aug. 8, 1960):										
Aug. 5, 1960.....	71.4	68.2	4.9	904	114	9.4	408	none	280	1.5
Aug. 16, 1960.....	68.6	68.2	4.2	1,025	114	9.1	463	none	348	1.1
Sept. 7, 1960.....	64.0	63.7	7.9	--	--	--	--	--	--	--
Aug. 9, 1961.....	71.0	70.0	4.3	678	84	9.2	336	trace	164	1.0
Raleigh Reservoir (treated Aug. 4, 1960):										
Aug. 4, 1960.....	72.1	70.5	3.2	266	184	8.4	153	none	86	1.0
Aug 15, 1960.....	71.7	68.8	4.7	336	196	8.7	143	none	96	0.5
Sept. 6, 1960.....	68.0	67.6	8.7	--	--	--	--	--	--	--
Aug. 8, 1961.....	71.0	68.2	11.0	380	180	9.8	200	28	143	0.5
South Lake Metigoshe (treated July 17, 1960):										
July 14, 1960.....	71.8	68.5	7.9	270	228	8.8	224	none	27	0.2
July 21, 1960.....	72.0	68.4	8.4	279	222	8.5	214	none	53	0.3
Aug. 26, 1960.....	64.6	62.3	8.3	--	--	--	--	--	--	--
Sept. 15, 1960.....	60.2	59.1	6.4	--	--	--	--	--	--	--
July 19, 1961.....	68.5	66.8	10.0	299	208	9.4	216	28	44	0.8
North Lake Metigoshe (untreated control):										
July 14, 1960.....	71.8	66.8	8.5	281	232	8.6	224	none	33	0.9
July 21, 1960.....	72.3	68.0	8.8	282	226	8.2	214	none	53	0.2
Aug. 26, 1960.....	64.9	61.9	6.4	--	--	--	--	--	--	--
Odland Reservoir (treated Aug. 11, 1960):										
Aug. 10, 1960.....	69.1	66.1	2.2	414	184	8.2	187	none	165	0.2
Aug. 16, 1960.....	68.0	65.8	1.8	510	202	8.2	195	none	186	0.8
Sept. 8, 1960.....	65.0	64.0	3.1	--	--	--	--	--	--	--
Aug. 8, 1961.....	71.2	68.0	2.1	574	208	8.5	196	trace	259	0.5

WOLF BUTTE RESERVOIR

DESCRIPTION

Wolf Butte Reservoir, in southwestern North Dakota, has a surface area of 24 acres and a maximum depth of 9 feet. It has no permanent inlet or outlet, and water is supplied mainly by runoff. The bottom is muck. No marked thermal stratification was present. The area surrounding the reservoir is primarily rangeland. Aquatic vegetation was very abundant at all depths less than 4 feet. Potamogeton pectinatus, P. richardsoni, and Myriophyllum exalbescentis, were the dominant plants. A heavy mat of filamentous algae (Rhizoclonium) accompanied these plants at the water surface.

TREATMENT

Fish.--Toxaphene was applied at 35 p.p.b. on August 8, 1960, in an attempt to eradicate the fish population. This impoundment was heavily populated with green sunfish (Lepomis cyanellus) black bullheads (Ictalurus melas), and a few large rainbow trout (Salmo gairdneri). Many green sunfish and black bullheads were found dead and dying after treatment. The reservoir was test-netted 1 week after eradication and again the following spring. Two 125-foot experimental gill nets were set for 24 hours, and no fish of any species were taken. The reservoir was test-netted again in August of 1961, when one 125-foot gill net and one frame net were set for 24 hours. The nets contained approximately 475 black bullheads and 83 trout. Many young-of-the-year green sunfish were also observed. A trapping program later in the fall revealed several adult green sunfish.

Plankton.--Four collections of plankton were made at one station near the center of the reservoir. Collections were made 3 days before treatment, and after treatment at 8 days, 30 days, and 366 days. The kinds and numbers of plankton are given for each collection in table 2. These are arranged in a phylogenetic order with the zooplankters first.

Comparison in numbers per liter was made between pretreatment and posttreatment collections. Rotifers were represented by nine genera, Keratella and Asplanchna being the

TABLE 2.--Number of plankters per liter in Wolf Butte Reservoir before and after toxaphene treatment at 35 parts per billion.

[Treated Aug. 8, 1960]

Organisms	Before Aug. 3, 1960	After		
		Aug. 16, 1960	Sept. 7, 1960	Aug. 9, 1961
Brachionus....	1	4	--	3
Keratella....	91	15	2	1
Lecane.....	--	--	--	1
Trichocerca...	1	--	--	--
Chromogaster..	1	2	1	--
Asplanchna....	73	106	--	--
Polyarthra....	7	13	1	2
Filinia.....	1	1	--	1
Hexarthra....	3	21	3	--
Daphnia.....	244	18	129	28
Simocephalus..	--	--	1	--
Ceriodaphnia..	4	9	18	--
Bosmina.....	98	130	18	25
Chydorus.....	1	--	--	--
Diaptomus....	6	--	--	2
Cyclops.....	46	3	11	13
Nauplii.....	106	26	10	73
Pandorina....	3	15	--	--
Oedogonium...	3	4	--	--
Cladophora....	--	--	--	² tr
Rhizoclonium..	4	1	8	1
Pediastrum...	12	5	3	61
Coelastrum...	--	--	--	3
Oocystis.....	1	3	--	--
Closteriopsis.	1	4	--	tr
Tetradon.....	--	1	--	--
Scenedesmus...	7	14	7	--
Mougeotia....	--	--	1	--
Spirogyra....	1	1	52	--
Closterium...	1	4	1	tr
Cosmarium....	4	1	2	--
Staurastrum...	3	3	--	--
Desmidium....	65	84	7	tr
Botryococcus..	--	3	2	7
Diatoma.....	2	1	1	2
Navicula.....	5	6	--	--
Pinnularia....	1	--	--	--
Pleurosigma...	--	--	1	--
Cymbella.....	1	tr	--	--
Nitzschia....	11	9	4	tr
Campliodiscus.	1	--	--	--
Ceratium.....	5	2	--	11
Synechocystis.	90,067	589,405	149,306	82,563
Polycystis....	242	131	2	110
Merismopedia..	--	--	1	--
Coelosphaerium.	50	28	1	--
Lyngbya.....	8	2	9	--
Anabaena.....	61	58	1	--
Aphanizomenon.	7,377	33,157	54,716	138
Nodularia.....	--	12	1	--

¹ Includes nauplii of both Diaptomus and Cyclops.

² Less than 1 per liter.

most numerous. Keratella changed from 91 before treatment to 15 at 1 week, 2 at 1 month, and only 1 at 1 year after treatment. Asplanchna increased from 73 before treatment to 106 at 1 week after treatment, but none were present in collections at 1 month or 1 year after treatment. Other rotifers were too scarce for comparisons.

Cladocerans were the most abundant zooplankters, with Daphnia and Bosmina appearing in large numbers. Daphnia decreased from 244 before treatment to 18 at 1 week, then increased to 129 at 1 month after treatment. Bosmina exhibited the reverse effect, and both were less abundant at 1 year after treatment. Copepoda were represented by Diaptomus, Cyclops, and undetermined nauplii. Six Diaptomus were taken before treatment, but none were found at 1 week or 1 month after treatment and only 2 at 1 year.

Cyclops decreased from 46 in the pretreatment collection to 3 at 1 week after treatment, but increased to 11 at 1 month. Nauplii decreased from 106 before treatment to 26 and 10 at 1 week and 1 month after treatment. Cyclops and nauplii were relatively abundant the following year.

There were 16 genera of Chlorophyta, 8 of Chrysophyta, and 1 of Pyrrophyta in the collections. None exhibited numerical changes which could be attributed to toxaphene treatment. Eight genera of Cyanophyta were present, and these were the most numerous algae. Synechocystis and Aphanizomenon were the most abundant genera. Synechocystis increased from 90,067 before treatment to 589,405 at 1 week after treatment, then decreased to 149,306 at 1 month. Aphanizomenon increased from 7,377 before treatment to 54,716 at 1 month after treatment. Polycystis, Coelospharium, and Anabaena decreased after treatment. Polycystis was abundant at 1 year, but Coelospharium and Anabaena did not reappear 1 year after treatment.

Most of the changes before and after treatment were small and could well be the result of normal fluctuations in the population or the result of sampling techniques. A few of these changes may have resulted from the toxaphene, but none were obvious.

Plant-inhabiting organisms.--Aquatic-plant-inhabiting organisms were collected at two stations on the same dates plankton was sampled. The numbers of organisms per pound of vegetation for the four collections is presented in table 3. Nineteen genera were represented, but only seven were numerous. Gammarus varied throughout the study, but remained abundant. Callibaetis, Caenis, and Ischnura decreased at 1 week and 1 month after treatment, but were more abundant at 1 year. Tendipes decreased from 44 before treatment to 9 at 1 week after treatment, while 48 were taken at 1 month and 25 at 1 year after treatment. Gastropoda (Physa and Gyraulus) increased from 771 before treatment to 1,107 at 1 week, 1,366 at 1 month, and 1,558 at 1 year after treatment.

TABLE 3.--Number of plant-inhabiting organisms and bottom fauna in Wolf Butte Reservoir before and after toxaphene treatment at 35 parts per billion.

[Plant-inhabiting organisms are expressed as the number per pound of plants and bottom fauna as the number per square foot of bottom. Treated Aug. 8, 1960. tr = less than 1 per pound or per square foot.]

Organism	Before Aug. 5, 1960		After					
			Aug. 16, 1960		Sept. 7, 1960		Aug. 9, 1961	
	Plant	Bottom	Plant	Bottom	Plant	Bottom	Plant	Bottom
Oligochaeta...	--	tr	--	tr	--	tr	--	3
Hirudinea.....	--	--	tr	--	--	--	--	--
Amphipoda:								
Gammarus.....	63	6	172	tr	73	44	265	tr
Hydracarina:								
Hydrachnidae..	5	--	tr	--	2	--	23	--
Ephemeroptera:								
Callibaetis..	5	1	tr	--	--	--	124	tr
Caenis.....	6	3	1	tr	--	tr	16	tr
Odonata:								
Sympetrum....	tr	--	tr	tr	--	--	3	--
Aeschna.....	--	--	tr	--	--	--	--	--
Ischnura.....	40	5	10	1	--	4	56	tr
Hemiptera:								
Plea.....	--	--	tr	--	--	--	--	--
Notonecta....	1	--	--	--	3	--	10	--
Sigara.....	3	--	--	--	tr	--	2	--
Coleoptera:								
Halipius....	2	--	tr	--	--	--	1	--
Copelatus....	tr	--	--	--	tr	--	2	--
Hydroporus..	--	--	--	--	1	--	tr	--
Trichoptera:								
Hydroptila..	--	1	--	tr	--	--	--	--
Diptera:								
Tendipes.....	44	28	9	12	48	9	25	25
Probezzia....	tr	3	--	2	--	--	--	--
Chrysops....	--	1	--	1	--	--	--	--
Gastropoda:								
Physa.....	123	2	325	1	156	3	886	6
Gyraulus....	648	tr	782	1	1,210	9	672	1
Pelecypoda:								
Pisidium.....	--	3	--	1	--	8	--	4

Numerical comparisons of the seven dominant genera revealed no marked changes before and after treatment. Reductions of Ephemeroptera and Odonata in the first two posttreatment collections may be significant but could have resulted from an emergence.

Bottom fauna.--These organisms were collected at the same stations as those used for plant-inhabiting organisms. Each collection consisted of 3 square feet of bottom. The number of organisms per square foot of bottom is given for each collection (table 3). Thirteen genera were taken, but only Gammarus and Tendipes were abundant. Gammarus fluctuated from 6 before treatment to less than 1 at 1 week, 44 at 1 month, and less than 1 at 1 year after treatment. The large number at 1 month after treatment resulted from a collection that contained considerable vegetation. Tendipes decreased from 28 before treatment to 12 and 9 at 1 week and 1 month after treatment but increased to 25 at 1 year. A comparison of the number of bottom organisms before and after treatment revealed no marked changes.

RALEIGH RESERVOIR

DESCRIPTION

Raleigh Reservoir, in southwestern North Dakota, has a surface area of 15 acres and a maximum depth of 18 feet. There are no permanent inlets or outlets, and the water is supplied mainly by runoff. The bottom is muck, covered by silt in some areas. No marked thermal stratification was present. The surrounding area is almost entirely rangeland. Aquatic vegetation was very abundant at all depths less than 3 feet. Potamogeton pectinatus, P. richardsoni, Myriophyllum exalbescens, and Ceratophyllum demersum were the dominant plants. Large amounts of filamentous algae (Rhizoclonium) accompanied these plants in most areas.

TREATMENT

Toxaphene was applied at 25 p.p.b. on August 4, 1960, in an attempt to remove the entire fish population. A complete kill was not achieved and a second treatment was made at 90 p.p.b. on September 26, 1960.

Fish.--Before treatment, two 125-foot experimental gill nets and four frame nets were set for 24 hours. The frame nets contained several thousand golden shiners (Notemigonus crysoleucas), approximately 5,000 green sunfish, and 1,200 white crappies (Pomoxis annularis) and black crappies (Pomoxis nigromaculatus). The two experimental gill nets captured 13 white suckers (Catostomus commersoni), 11 black bullheads, and a few golden shiners, green sunfish, and crappies. Large numbers of the four most numerous species were found dead and dying after treatment. The reservoir was netted again 1 week after the first treatment, but with only two experimental gill nets set for 24 hours. These contained 10 white suckers and 5 black bullheads. Test-netting was discontinued since drought had lowered water levels to a point where restocking was impracticable.

Plankton.--Four collections were made at one station near the center of the reservoir. Collections were made 1 day before the first treatment and at 11, 33, and 371 days after the

first treatment; a second treatment was made 53 days after the first, and the fourth collection was 318 days after this treatment. Quantities of plankters in pretreatment and posttreatment collections are shown in table 4. Rotifers were represented by 15 genera, but only Brachionus and Asplanchna were abundant. Brachionus decreased from 114 before treatment to 108 at 11 days and 15 at 33 days after treatment. Asplanchna varied from 24 before treatment to 194 at 11 days and 16 at 33 days after treatment. Only 3 Brachionus and 1 Asplanchna were

TABLE 4.--Number of plankton per liter in Raleigh Reservoir before and after treatment at 25 parts per billion.
[Treated Aug. 4, and Sept. 26, 1960]

Organism	Before Aug. 3, 1960	After		
		Aug. 15, 1960	Sept. 6, 1960	Aug. 10, 1961 ¹
Brachionus...	114	108	15	3
Keratella....	13	11	9	7
Platylas.....	2 tr	17	1	--
Lecane.....	tr	2	--	--
Monostyla....	1	1	--	5
Trichocerca..	5	--	--	tr
Chromogaster.	1	3	--	1
Asplanchna...	24	194	16	1
Polyarthra...	3	15	7	1
Synchaeta....	1	11	13	--
Filinia.....	tr	1	--	tr
Testudinella.	1	--	tr	--
Trochosphaera	tr	--	1	--
Hexarthra....	2	--	--	--
Conochiloides	3	11	--	--
Daphnia.....	65	173	57	9
Ceriodaphnia.	44	156	100	1
Bosmina.....	314	283	50	--
Chydorus.....	4	17	1	--
Diaptomus....	10	1	1	--
Cyclops.....	120	9	41	8
Nauplii ²	190	85	19	7
Elakothrix...	--	--	1	--
Microspora...	--	1	3	--
Oedogonium...	9	3	1	--
Rhizoclonium.	7	5	3	1
Golenkinia...	--	3	--	--
Pediastrum...	151	462	3	3
Coelastrum...	7	594	--	--
Oocystis.....	69	75	2	--
Chodatella...	4	15	--	--
Closteriopsis	18	462	1	--
Tetraedon....	11	89	--	--
Scenedesmus..	727	3,038	133	1
Crucigenia...	17	264	4	--
Tetrastrum...	--	3	--	--
Mougeotia....	--	1	1	--
Zygnema.....	1	--	--	--
Spirogyra....	2	4	19	107
Closterium...	--	--	2	tr
Cosmarium....	20	4	5	1
Staurostrum..	4	9	--	--
Desmidiium...	925	1,189	4	--
Botryococcus.	5	8	7	--
Melosira.....	4	9	--	2
Diatoma.....	8	--	3	1
Synedra.....	4	3	--	3
Navicula.....	6	1	1	--
Pinnularia...	1	--	1	--
Frustulia....	--	tr	1	--
Gyrosigma....	--	tr	--	--
Pleurosigma...	tr	--	--	--
Gomphonema...	tr	1	--	--
Cymbella.....	4	4	1	--
Nitzschia....	8	14	7	8
Cymatopleura.	2	1	--	--
Campylodiscus	2	--	--	--
Ceratium.....	24	7	4	5
Synechocystis	54,161	6,275	2,312	601,057
Polycystis...	2,906	859	38	99
Merismopedia.	9	7	1	--
Coelosphaerium	2	25	94	4
Lyngbya.....	10	1	8	1
Anabaena.....	5	4	16	2
Aphanizomenon	6	190	81,902	tr
Nodularia....	77	3,633	21	3

¹ After the second treatment at 90 p.p.b.

² Less than 1 per liter.

³ Includes nauplii of both Diaptomus and Cyclops.

taken 371 days after treatment. All rotifers were very scarce at this time, and 6 of the original genera were not found.

Cladocera was the most abundant zooplankter. Daphnia, Ceriodaphnia, and Bosmina were present in large numbers. Daphnia varied from 65 before treatment to 173 at 11 days, 57 at 33 days, and only 9 at 371 days after treatment. Ceriodaphnia increased from 44 before treatment to 156 at 11 days, then decreased to 100 at 33 days, and only 1 was taken at 371 days after treatment. Bosmina decreased from 314 before treatment to 283 at 11 days and 50 at 33 days after treatment and disappeared by 371 days. A few Chydorus were found in the pretreatment and early posttreatment collections, but did not occur in the collection 371 days after treatment. Copepoda were represented by the young and adults of Diaptomus and Cyclops. Diaptomus changed from 10 before treatment to 1 at 11 days and 1 at 33 days, but none at 371 days after treatment. There were 120 Cyclops before treatment while collections after treatment showed 9 at 11 days, 41 at 33 days, and 8 at 371 days. Nauplii decreased from 190 before treatment to 85 at 11 days, 19 at 33 days, and 7 at 371 days after treatment.

The Chlorophyta were represented by 21 genera. Pediastrum, Coelastrum, Closteriopsis, Tetraedon, Scenedesmus, Crucigenia, and Desmidium were the most abundant. All of these increased in the collection 11 days after treatment but were greatly reduced at 33 days and 371 days. Spirogyra was the most abundant of the Chlorophyta in the collection 371 days after treatment, but was scarce in the pretreatment and early posttreatment collections. The Chrysophyta contained 9 genera and the Pyrrophyta 1. These were infrequently encountered, and no comparisons were made. The Cyanophyta were represented by 8 genera. Synechocystis, Polycystis, Coelospharium, Aphanizomenon, and Nodularia were the dominant organisms. Synechocystis and Polycystis decreased in the first two posttreatment collections but were abundant at 371 days after treatment. Coelospharium and Aphanizomenon increased after treatment but were scarce at 371 days after treatment. Nodularia varied from 77 before treatment to 3,663 at 11 days, 21 at 33 days, and 3 at 371 days after treatment.

Changes after the first treatment (25 p.p.b.) are probably the result of normal population fluctuations. At 371 days after treatment water levels had dropped approximately 6 feet, the water was clear, and aquatic vegetation had increased. The severe reduction in nearly all plankters at this time may have been due to the drop in water levels or the possible consequent increased toxaphene concentration.

Plant-inhabiting organisms.--Collections were made at two stations on the same dates plankton was collected. The number of organisms per pound of vegetation is presented for each collection (table 5). Nineteen genera were taken, but only eight were abundant. Gammarus increased from 31 before treatment to 313 at 11 days, 569 at 33 days, and 334 at 371 days after treatment. Hydrachnidae decreased from 45 before treatment to 27 at 11 days, 22 at 33 days, and 14 at 371 days after treatment. Callibaetis, Caenis, Ischnura, and Tendipes were markedly reduced in the first two posttreatment collections, but all except Caenis were abundant at 371 days after treatment. Sigara decreased from 39 before treatment to less than 1 at 11 days after treatment, and none were taken after

TABLE 5.--Numbers of plant inhabiting organisms and bottom fauna in Raleigh Reservoir before and after toxaphene treatment at 25 parts per billion.

[Plant inhabiting organisms are expressed as the number per pound of plants and bottom fauna as the number per square foot of bottom. Treated Aug. 4, and Sept. 26, 1960.]

Organism	Before Aug. 3, 1960		After					
	Plant	Bottom	Aug. 15, 1960	Bottom	Sept. 6, 1960	Bottom	Aug. 10, 1961 ¹	Bottom
Oligochaeta...	--	4	--	8	--	11	--	28
Hirudinea.....	2 tr	--	tr	--	--	--	5	--
Amphipoda:								
<u>Gammarus</u>	31	4	313	tr	569	3	334	1
Hydracarina:								
Hydrachnidae:	45	--	27	--	22	--	14	--
Ephemeroptera:								
<u>Callibaetis</u> ..	66	1	9	tr	tr	--	60	--
<u>Caenis</u>	185	6	10	tr	6	--	3	--
Odonata:								
<u>Sympetrum</u>	--	--	1	--	tr	--	17	--
<u>Anax</u>	--	--	--	--	--	--	1	--
<u>Aeschna</u>	tr	--	tr	--	--	--	1	--
<u>Ischnura</u>	109	2	21	1	8	--	134	--
Hemiptera:								
Notonecta....	2	--	tr	--	tr	--	5	--
<u>Sigara</u>	39	2	tr	--	--	--	--	--
Coleoptera:								
<u>Copelatus</u>	--	--	tr	--	--	--	--	--
<u>Hydroporus</u> ...	2	--	7	--	6	--	8	--
Diptera:								
<u>Chaoborus</u>	--	1	--	--	--	--	--	--
<u>Tendipes</u>	12	27	tr	2	1	6	52	71
<u>Probezzia</u>	tr	--	tr	--	--	--	--	--
<u>Chrysops</u>	--	--	tr	--	--	tr	--	--
Gastropoda:								
<u>Physa</u>	5	--	13	--	6	--	22	--
<u>Gyraulus</u>	1,163	tr	1,695	4	398	2	38	--
Pelecypoda:								
<u>Pisidium</u>	--	tr	--	4	--	7	--	--

¹ After the second treatment at 90 p.p.b.

² Less than one per pound or square foot.

this time. There were 1,163 Gyraulus before treatment, 1,695 at 11 days, 398 at 33 days, and 38 at 371 days after treatment.

Several changes were noted following treatment, some of which may be the result of the toxaphene. Reductions of Callibaetis, Caenis, Ischnura, and Tendipes may be significant; all but Caenis, however, were abundant 371 days after treatment. Stringer and McMynn (1958) reported that Ephemeroptera were killed at 30 p.p.b. toxaphene. The disappearance of Sigara after treatment appears to be the result of the toxaphene since they exhibited low tolerance levels in the controlled experiments (table 11). The reduction of Gyraulus at 371 days after treatment may be related to lowered water levels, since other workers (Hooper and Grzenda, 1957; and Stringer and McMynn, 1958) found Gastropoda to be unaffected by toxaphene at 100 p.p.b.

Bottom fauna.--Four collections were made at two stations on the same dates plant-inhabiting organisms were collected. The number per square foot of bottom is given for each collection (table 5). Eleven genera were taken, but most of these were too scarce for comparisons. Oligochaeta increased throughout the study from 4 before treatment to 28 at 371 days after treatment. Cushing and Olive (1957) found an increase in Oligochaeta after treatment with 100 p.p.b. toxaphene. Ephemeroptera decreased from 7 before treatment to less than 1 at 11 days, and none were taken in succeeding collections. Tendipes decreased from 27 before treatment to 2 at 11 days and 6 at 33 days after treatment, then increased to 71 at 371 days after treatment.

The reductions of Ephemeroptera and Tendipes may be significant. Stringer and McMynn (1958) reported that Ephemeroptera were killed at 30 p.p.b. of toxaphene, and Cushing and Olive found that a concentration of 100 p.p.b. eliminated Tendipedidae.

SOUTH LAKE METIGOSHE

DESCRIPTION

South Lake Metigoshe is a glacial lake in the Turtle Mountains in north central North Dakota.

It has an area of 915 surface acres and an average depth of 9 feet. Water is supplied mainly by runoff. Water levels fluctuate slightly owing to releases from an upstream reservoir. The major bottom materials are peat and muck. No marked thermal stratification was present. Trees border most of the shoreline. Aquatic vegetation was common and was exceptionally abundant in the bays. Scirpus sp. occupied several large areas near shore. Myriophyllum exallescens and Ceratophyllum demersum were present at most depths less than 15 feet. Other dominant plants were Potamogeton natans, P. pectinatus, P. richardsoni, P. zosteriformis, Najas flexilis, Sagittaria latifolia, Eleocharis palustris, and Polygonum amphibium.

TREATMENT

Toxaphene was applied at 10 p.p.b. on July 17, 1960, in an attempt to reduce the number of yellow perch (Perca flavescens) and black bullheads. This was supplemented by 5 p.p.b. on July 19.

Fish.--Several 250-foot experimental gill nets and frame nets (0.5-inch and 0.25-inch mesh) were set at selected stations 1 week before, 1 week after, and again 11 months after treatment; the netting efforts were 333, 290, and 120 hours, respectively. The fish taken are expressed as the number per 100 net-hours. Adult yellow perch were reduced from 900 before treatment to 36 at 1 week and none at 11 months after treatment. Young-of-the-year were reduced from 610 before treatment to 6 at 1 week and none at 1 year after treatment. Young-of-the-year black bullheads decreased from 240 before treatment to 9 at 1 week and none at 11 months after treatment. Young-of-the-year northern pike (Exos lucius) decreased from 40 before treatment to 10 at 1 week and none at 11 months after treatment. Netting at 1 week after treatment did not show a reduction in adult black bullhead, northern pike, and walleye (Stizostedion vitreum), but several were found dead along shore at this time. No walleye were taken at 11 months after treatment, and bullheads and northern pike were greatly reduced. The paucity of all species taken at 11 months after treatment may have been due to the residual effects of the toxaphene.

Plankton.--Five collections were made at four stations on South Lake Metigoshe. These were made 2 days before treatment and at 4, 40, 60, and 367 days after treatment. The pre-treatment and the first two posttreatment collections at South Lake Metigoshe are compared with those made at four stations on North Lake Metigoshe, which was sampled on the same

dates. North Lake Metigoshe is adjacent to South Lake Metigoshe and is connected by a channel approximately 30 feet wide; it was not treated until later in the fall and could therefore be used as a control. The number of plankton per liter for all collections in both lakes is given in table 6.

TABLE 6.--Number of plankton per liter in South and North Lake Metigoshe before and after toxaphene treatment at 15 parts per billion

[South Lake Metigoshe treated July 17, 1960]

Organism	Before, July 15, 1960		After -					
	South	North	July 21, 1960		Aug. 26, 1960		Sept. 15, 1960, South	July 19, 1961, South
			South	North	South	North		
Brachionus.....	--	2	1	1	¹ tr	--	1	--
Keratella.....	35	28	48	22	1	25	11	12
Lecane.....	tr	--	--	--	1	tr	--	tr
Monostyla.....	3	--	2	--	9	2	1	2
Trichocerca.....	234	111	83	86	31	5	3	3
Ascomorpha.....	1	3	5	4	4	tr	tr	1
Chromogaster.....	tr	2	tr	2	3	--	tr	1
Asplanchna.....	28	3	24	6	1	12	--	20
Polyarthra.....	4	27	35	34	42	32	28	16
Synchaeta.....	17	1	19	3	10	2	1	tr
Filinia.....	3	3	25	2	7	2	2	3
Testudinella.....	--	--	--	tr	--	--	--	tr
Hexarthra.....	11	1	15	2	--	1	--	tr
Conochiloides.....	182	14	160	34	1	10	--	tr
Stephanoceros.....	--	--	--	--	--	tr	--	--
Daphnia.....	1	9	1	2	9	5	16	41
Simocephalus.....	1	--	2	--	1	1	tr	tr
Ceriodaphnia.....	37	99	67	37	40	18	7	1
Bosmina.....	110	67	119	35	8	18	8	1
Graptolebris.....	--	--	--	--	4	--	--	--
Chydorus.....	10	1	2	tr	20	3	19	5
Diaptomus.....	1	12	tr	13	tr	5	--	2
Cyclops.....	17	17	4	17	13	9	22	44
Nauplii ²	29	83	17	76	28	37	11	67
Pandorina.....	1	tr	1	1	5	5	5	5
Volvox.....	tr	tr	1	1	15	1	1	--
Apicocystis.....	--	--	--	--	--	--	--	tr
Oedogonium.....	--	1	tr	tr	3	--	tr	--
Rhizoclonium.....	tr	tr	tr	tr	--	--	--	--
Pediastrum.....	4	17	2	7	7	38	6	6
Coelastrum.....	tr	tr	tr	tr	--	--	1	--
Oocystis.....	1	1	1	1	1	tr	--	2
Chodatella.....	--	tr	tr	--	--	--	--	tr
Closteriopsis.....	1	2	1	2	2	tr	1	5
Kirchneriella.....	--	tr	--	--	--	--	--	--
Tetradon.....	--	--	tr	--	--	--	--	--
Scenedesmus.....	tr	1	1	1	1	2	tr	1
Crucigenia.....	tr	tr	8	--	1	--	1	1
Mougeotia.....	--	--	--	--	--	--	tr	tr
Spirogyra.....	tr	1	tr	--	1	--	tr	tr
Closterium.....	tr	--	tr	--	--	--	--	--
Cosmarium.....	1	tr	1	tr	--	3	1	tr
Staurastrum.....	15	16	17	8	60	23	106	67
Desmidiium.....	3	4	4	1	2	3	1	1
Botryococcus.....	--	1	--	2	2	tr	2	2
Dinobryon.....	7	98	1	11	--	tr	--	--
Melosira.....	11	73	5	40	568	1,040	462	3
Diatoma.....	1	1	--	1	--	--	1	1
Fragilaria.....	144	235	71	45	871	50	71	3,078
Synedra.....	42	51	60	13	304	62	581	6,803
Asterionella.....	1	1	1	tr	tr	1	1	tr
Naviacula.....	13	1	22	1	25	29	3	6
Pinnularia.....	4	1	16	1	5	8	1	1
Frustulia.....	1	--	1	--	1	1	--	--
Gyrosigma.....	tr	--	tr	--	--	1	tr	tr
Pleurosigma.....	tr	tr	--	--	1	tr	tr	tr
Gomphonema.....	2	9	9	6	39	9	61	61
Cymbella.....	4	1	18	1	10	2	1	1
Nitzschia.....	2	2	19	4	7	3	3	12
Cymatopleura.....	--	--	--	--	--	tr	1	--
Camplodydiscus.....	2	tr	1	tr	3	1	1	tr
Glenodinium.....	tr	1	1	2	--	1	--	--
Ceratium.....	52	42	71	61	190	300	260	948
Polycystis.....	87	396	73	330	155	1,057	218	462
Merismopedia.....	--	--	--	tr	--	--	--	1
Coelosphaerium.....	7	132	9	238	150	2,510	470	207
Phormidium.....	3	--	4	--	1	--	tr	--
Lyngbya.....	13	24	21	27	2,312	2,932	81	39
Anabaena.....	7,794	3,540	1,982	1,915	130,515	4,716	127,470	1,222
Aphanizomenon.....	2,642	5,773	1,096	1,836	69,617	4,742	98,018	53
Nodularia.....	3	2	14	4	6,869	4	5,019	39
Gloeotrichia.....	3	1	3	1	2	--	1	1

¹ Represents less than one per liter.

² Includes nauplii of *Diaptomus* and *Cyclops*.

Rotifers were represented by 15 genera. Trichocerca and Conochiloides were the most abundant. Trichocerca decreased from 234 before treatment to 31 at 40 days, 3 at 60 days, and 3 at 367 days after treatment. Conochiloides decreased from 182 before treatment to 160 at 1 day, 1 at 40 days, none at 60 days, and less than 1 at 367 days after treatment. Asplanchna decreased from 28 before treatment to 1 at 40 days, none at 60 days, and 20 at 367 days after treatment. Hexarthra decreased from 11 before treatment to none at 40 days, none at 60 days, and less than 1 at 367 days after treatment. Keratella, Polyarthra, and Filinia remained almost constant. There were six genera of Cladocera; Daphnia, Ceriodaphnia, and Bosmina were the most abundant. Daphnia increased from 1 before treatment to 41 at 367 days after treatment; Ceriodaphnia remained nearly constant, but was less abundant at 367 days after treatment; Bosmina decreased from 110 before treatment to 8 at 40 days and 1 at 367 days after treatment. Copepoda was an abundant group, represented by Diaptomus, Cyclops, and their nauplii, which remained nearly constant during the study.

There were 20 genera of Chlorophyta, 2 of Pyrrophyta, and 17 of Chrysophyta. Staurostrum, Ceratium, Melosira, Fragilaria, and Synedra were the dominant organisms in these groups. After treatment these increased at 40 days after treatment and, with the exception of Melosira, were more abundant at 367 days after treatment. Cyanophyta were the most abundant phytoplankters. Polycystis, Coelospharium, Lyngbya, Anabaena, Aphanizomenon, and Nodularia were abundant. Polycystis and Coelospharium increased slightly after treatment, whereas Anabaena, Lyngbya, Aphanizomenon, and Nodularia exhibited large post-treatment increases (table 6).

Comparisons of zooplankters before and after treatment revealed no marked changes. Post-treatment decreases in South Lake Metigoshe were not significant, because comparisons with untreated North Lake Metigoshe revealed similar decreases in most instances during the same period. Most of the dominant phytoplankters remained almost constant or in-

creased following treatment. The large post-treatment increase exhibited by Anabaena, Aphanizomenon, and Nodularia may be the result of treatment, since they did not increase significantly in North Lake Metigoshe.

Plant-inhabiting organisms.--Four stations were established on South Lake Metigoshe and five collections were made at each of these. Collections were made 1 day before treatment, and at 5, 45, 59, and 367 days after treatment. The pretreatment and the first two posttreatment collections at South Lake Metigoshe are compared with three collections made at four stations on North Lake Metigoshe, which were not treated until later in the fall. The number of organisms per pound of plants is given for both South and North Lake Metigoshe (table 7, p. 12). There were 28 genera of organisms taken; Gammarus, Tendipes, Physa, and Gyraulus were the most abundant. A comparison of pretreatment and posttreatment collections revealed no marked changes in the number of any organism.

Bottom fauna.--No pretreatment samples were obtained, but two posttreatment samples were taken, which consisted of 3 square feet each. Tendipes was the dominant organism, but a few Gammarus, Chaoborus, and Oligochaeta were also present. Nine Tendipes per square foot were taken at 60 days and 31 at 367 days after treatment.

ODLAND RESERVOIR

DESCRIPTION

Odland Reservoir, in southwestern North Dakota, has a surface area of 100 acres and a maximum depth of 16 feet. There are no permanent inlets or outlets, and the water is supplied mainly by runoff. The major bottom material is muck. No marked thermal stratification was present. Aquatic vegetation was abundant in all shallow areas. Potamogeton pectinatus, P. richardsoni, Scirpus sp., Myriophyllum exalbescens, and Chara sp. were the dominant plants.

TABLE 7.--Number of plant-inhabiting organisms and bottom fauna in South and North Lake Metigoshe before and after toxaphene treatment at 15 parts per billion.

[Plant inhabiting organisms expressed as the number per pound of plants and bottom fauna as number per square foot of bottom.
South Lake Metigoshe treated July 17, 1960]

Organism	Before, July 16, 1960		After					
	South	North	July 22, 1960		Aug. 31, 1960		Sept. 16, 1960, South	July 18, 1961, South
			South	North	South	North		
Hirudinea.....	1	¹ tr	tr	tr	1	tr	1	2
Amphipoda:								
<i>Gammarus</i>	639	198	406	186	177	130	295	424
Hydracarina:								
Hydracnidae.....	11	4	5	4	1	1	1	1
Ephemeroptera:								
<i>Callibaetis</i>	2	tr	--	tr	--	1	tr	6
<i>Caenis</i>	1	3	2	2	--	2	--	--
Odonata:								
<i>Sympetrum</i>	tr	--	--	--	--	--	--	--
<i>Anax</i>	tr	tr	--	tr	--	--	--	1
<i>Aeschna</i>	--	--	--	--	--	tr	--	1
<i>Ischnura</i>	tr	tr	tr	1	tr	7	tr	2
Hemiptera:								
<i>Plea</i>	tr	1	tr	tr	tr	2	tr	7
<i>Notonecta</i>	--	tr	--	--	--	--	--	3
<i>Buenos</i>	tr	--	--	--	--	--	tr	--
<i>Sigara</i>	tr	tr	tr	tr	tr	--	tr	6
Trichoptera:								
<i>Psychomyia</i>	1	1	--	1	--	tr	--	tr
<i>Oecetis</i>	1	1	tr	1	--	tr	--	--
<i>Triacnodes</i>	3	1	tr	tr	--	--	--	tr
<i>Phryganea</i>	tr	tr	tr	tr	--	tr	--	--
Coleoptera:								
<i>Copelatus</i>	2	2	1	3	tr	3	1	11
<i>Halipus</i>	1	2	tr	2	tr	2	tr	2
<i>Hydrocanthus</i>	--	tr	--	--	tr	--	tr	2
<i>Cyrtinus</i>	--	--	tr	--	--	tr	--	--
Diptera:								
<i>Chaoborus</i>	tr	--	1	--	--	--	--	--
<i>Tendipes</i>	9	5	3	6	11	3	2	1
<i>Probezzia</i>	tr	1	tr	tr	tr	--	--	--
Gastropoda:								
<i>Physa</i>	24	28	21	27	31	19	29	66
<i>Lymnaea</i>	--	tr	--	tr	--	--	tr	--
<i>Gyraulus</i>	47	56	24	44	51	105	86	10
<i>Valvata</i>	--	tr	tr	--	tr	--	tr	--

¹ Represents less than 1 per pound.

TREATMENT

Fish.--Toxaphene was applied at 5 p.p.b. on August 11, 1960, to reduce young-of-the-year black bullheads and yellow perch. Large numbers of these fish and several young-of-the-year northern pike, white crappie, and orange-spotted sunfish (*Lepomis humilis*) were found dead along shore the day after treatment. Adults of these species were not significantly reduced by treatment, since only a few were found dead and large numbers were taken in posttreatment test nettings.

Plankton.--Four collections were made at each of two stations. These were made 1 day before treatment, and at 7, 28, and 362 days after treatment. The kinds and number of plankters per liter are given for each collection (table 8). There were 10 genera of Rotifera taken; *Brachionus*, *Keratella*, *Polyarthra*, and *Conochiloides* were the most abundant and remained nearly constant before and after treatment. Cladocera were the most abundant zooplankters with *Daphnia*, *Ceriodaphnia*, and

Bosmina being most common. There were 22 *Daphnia* before treatment, 68 at 7 days, 40 at 28 days and 3 at 362 days after treatment. *Ceriodaphnia* varied from 31 before treatment to 66 at 7 days, 37 at 28 days, and only 1 at 362 days after treatment. *Bosmina* decreased from 459 before treatment to 24 at 28 days and 66 at 362 days after treatment. Copepoda were represented by adults and nauplii of *Diaptomus* and *Cyclops*. *Cyclops* decreased from 71 before treatment to 16 at 28 days after treatment, and nauplii decreased from 129 before treatment to 36 at 28 days after treatment, but both were again abundant at 362 days after treatment. Fourteen genera of Chlorophyta, 12 of Chrysophyta, 1 of Pyrrophyta, and 5 of Cyanophyta were found. *Melosira*, *Ceratium*, *Polycystis*, and *Aphanizomenon* were the dominant organisms of these groups. These increased at 7 days and 28 days after treatment. Approximately the same numbers were found in post-treatment collections at 362 days as were found before treatment. Numerical comparisons of pretreatment and posttreatment collections showed no marked changes.

TABLE 8.--Number of plankton per liter in Odland Reservoir before and after toxaphene treatment at 5 parts per billion.

[Treated Aug. 11, 1960]

Organism	Before, Aug. 10, 1960	After		
		Aug. 18, 1960	Sept. 8, 1960	Aug. 8, 1961
Brachionus...	--	10	26	12
Keratella....	3	7	71	33
Trichocerca...	--	1	1	--
Asplanchna...	5	3	6	4
Polyarthra...	7	61	55	229
Synchaeta....	1	2	17	4
Filinia.....	--	1	11	1
Trochosphaera	1	1	7	4
Hexarthra...	1 tr	tr	--	tr
Jonochiloides	--	tr	63	tr
Daphnia.....	22	68	40	3
Simoscephalus	--	--	tr	--
Ceriodaphnia.	31	66	37	1
Bosmina.....	59	333	24	66
Chydorus.....	--	--	2	--
Diaptomus....	1	1	1	--
Cyclops.....	71	26	16	34
Nauplii ²	129	61	36	115
Oedogonium...	--	1	--	--
Rhizoclonium.	--	--	3	--
Microactinium	--	1	--	tr
Pediastrum...	4	3	10	12
Hydrodictyon.	--	tr	--	--
Oocystis.....	tr	1	2	--
Closteropsis	10	5	1	2
Tetraodon....	tr	1	1	--
Scenedesmus..	5	3	7	1
Mougeotia....	--	--	1	--
Spirogyra....	--	tr	1	--
Closterium...	tr	--	1	1
Cosmarium....	1	2	tr	--
Staurastrum..	3	2	6	1
Desmidiun....	3	8	16	2
Botryococcus.	--	--	1	--
Dinobryon....	--	--	11	--
Melosira.....	6	55	33	131
Diatoma.....	2	2	1	16
Synedra.....	tr	1	1	4
Asterionella.	1	1	6	tr
Navicula.....	tr	1	1	--
Pleurosigma..	--	tr	--	--
Cymbella.....	1	tr	--	--
Nitzschia....	6	15	3	1
Cymatopleura.	tr	tr	--	tr
Ceratium.....	391	542	3,302	1,123
Polycystis...	6	9	130	3
Lyngbya.....	1	1	3	--
Anabaena.....	1	5	3	5
Aphanizomenon	14	287	5,812	4
Nodularia....	tr	1	8	--

¹ Represents less than one per liter.² Includes nauplii of both *Diaptomus* and *Cyclops*.

Plant-inhabiting organisms.--Four collections were made at two stations on the same dates plankton were collected. The number of organisms per pound of plants is given for each collection (table 9). Nineteen genera were taken, with *Gammarus*, *Hydrachnidae*, *Caenis*, *Ischnura*, *Tendipes*, *Physa*, *Gyraulus*, and *Valvata* being the most abundant. *Caenis* and *Tendipes* decreased slightly after treatment, probably a normal population fluctuation rather than a result of the toxaphene treatment. All other organisms remained nearly constant.

Bottom fauna.--Four collections were made at the same stations on the same dates plant inhabiting organisms were collected. Each sample contained 4 square feet of bottom. The number of organisms per square foot of bottom is given for each collection (table 9). Fourteen genera were taken, but only *Tendipes*, *Physa*,

TABLE 9.--Number of plant-inhabiting organisms and bottom fauna in Odland Reservoir before and after toxaphene treatment at 5 parts per billion.

[Plant-inhabiting organisms expressed as number per pound of plants and bottom fauna as number per square foot of bottom. Treated August 11, 1960]

Organism	Before, Aug. 10, 1960		After					
			Aug. 18, 1960		Sept. 8, 1960		Aug. 8, 1961	
	Plant	Bottom	Plant	Bottom	Plant	Bottom	Plant	Bottom
Oligochaeta...	--	2	--	1	--	¹ tr	--	2
Hirudinea.....	tr	--	tr	--	--	1	2	--
Amphipoda:								
<i>Gammarus</i>	195	1	294	2	303	6	76	tr
<i>Hydracarina</i> :								
<i>Hydrachnidae</i> .	53	--	34	--	4	--	17	--
<i>Ephemeroptera</i> :								
<i>Caenis</i>	21	2	11	tr	5	--	11	tr
<i>Odonata</i> :								
<i>Sympetrum</i>	1	--	tr	--	--	--	--	--
<i>Aeschna</i>	tr	tr	tr	--	--	--	2	--
<i>Ischnura</i>	5	1	4	tr	13	1	11	--
<i>Hemiptera</i> :								
<i>Notonecta</i>	tr	--	1	--	2	--	--	--
<i>Sigara</i>	tr	--	tr	--	1	--	--	--
<i>Trichoptera</i> :								
<i>Hydroptila</i> ..	--	1	--	--	--	--	--	--
<i>Psychomyia</i> ..	tr	--	--	--	--	--	--	--
<i>Phryganea</i>	tr	--	--	--	--	--	--	--
<i>Coleoptera</i> :								
<i>Halophilus</i>	6	--	1	--	1	--	tr	--
<i>Hydroporus</i> ..	tr	--	tr	--	2	--	--	--
<i>Diptera</i> :								
<i>Tendipes</i>	37	18	6	8	4	2	14	8
<i>Probezzia</i>	--	--	tr	--	--	tr	--	tr
<i>Chrysops</i>	--	tr	--	1	--	tr	--	--
<i>Gastropoda</i> :								
<i>Physa</i>	32	3	52	9	23	1	89	2
<i>Gyraulus</i>	1,301	7	964	11	721	17	320	2
<i>Valvata</i>	48	11	52	17	149	78	7	23
<i>Pelecypoda</i> :								
<i>Pisidium</i>	--	9	--	11	--	15	--	32

¹ Less than 1 per pound or square foot.

Gyraulus, *Valvata*, and *Pisidium* were abundant. None of these exhibited marked numerical changes that could be attributed to toxaphene treatment.

EXPERIMENTS

Six Rotifera, two Cladocera, and two Copepoda were tested at six toxaphene concentrations ranging from 50 to 1,000 p.p.b., to determine their tolerance levels. All tests were conducted in battery jars, each containing 8 liters of filtered lake water taken at the site where the organisms were collected. The water had an average temperature of 68° F., a dissolved oxygen content of 9.8 p.p.m., total alkalinity of 341 p.p.m., and pH of 8.4. Before each experiment the jars were washed with steel-wool soap pads and rinsed. All organisms were collected by pumping lake water through a No. 20 plankton net and were then placed in the jars. The toxaphene was diluted with water and applied to the water surface with moderate mixing, and after 24 hours the plankters were removed by siphoning into a No. 20 plankton net and concentrated to 25 cc. All organisms in 2 cc. of this sample were counted. To avoid collecting the dead and

affected plankters the jars were tilted and 200 cc. were left in the bottom after drainage. Three trials were conducted at each concentration, and the number of organisms counted was compared with untreated controls, which were maintained for all experiments (table 10).

Larger invertebrates were tested to determine the concentration at which 100 percent survived for 24 hours and 100 percent were killed (table 11). These tests were carried out in galvanized tanks, each containing 20 gallons of lake water at 71° F. The water used, toxaphene application, and cleaning method were the same as for zooplankters. In most cases 10 to 20 organisms were used to calculate percent survival and mortality. Controls were maintained for 2 weeks, then discontinued, and survival was assumed to be 100 percent, with the exception of Gammarus which showed 91 percent survival.

Fathead minnows (Pimephales promelas) approximately 1 inch in length were placed in all containers after washing, for 48 hours at 10-

day intervals. This was done to determine whether large amounts of toxaphene were accumulating because of inadequate washing, since these minnows were found to have low tolerance levels (Hooper and Grzenda, 1957). The lowest concentration used in the experiments was 10 p.p.b., which produced 100 percent mortality among the test fish while all experimental organisms survived. No fathead minnows died in the washed tanks, and it was assumed that the procedure was adequate.

RESULTS

Marked reductions of rotifers were first observed at 500 p.p.b., cladocerans (Daphnia pulex and Boxminia) at 250 p.p.b., and copepods at 100 p.p.b. (table 10). All genera in each group exhibited similar tolerance levels. Four trials employing 10 organisms each were conducted with Daphnia magna at six concentrations. No effects were obvious at 50 to 400 p.p.b., however retarded movements were observed at 1,000 p.p.b., and movements had nearly ceased at 1,500 p.p.b. Prevost (1960) reported a median

TABLE 10.--Comparison in number of zooplankters per cc. in 25-cc. concentrates from treated and control jars.

Organism and trial	50 p.p.b.		100 p.p.b.		250 p.p.b.		500 p.p.b.		750 p.p.b.		1,000 p.p.b.	
	Control	Treated	Control	Treated	Control	Treated	Control	Treated	Control	Treated	Control	Treated
Polyarthra:												
Trial 1.....	1	1	5	4	5	6	0	2	1	0	2	1
Trial 2.....	4	6	0	1	0	2	69	23	14	1	0	0
Trial 3.....	7	4	7	2	1	4	10	0	10	0	2	1
Hexarthra:												
Trial 1.....	4	5	5	5	5	2	0	0	4	1	0	0
Trial 2.....	111	133	14	6	10	10	8	0	111	2	4	1
Trial 3.....	3	4	3	2	4	1	7	1	7	0	20	1
Filinia:												
Trial 1.....	19	26	14	35	14	14	22	11	19	1	3	0
Trial 2.....	6	5	4	3	22	8	17	9	6	1	4	0
Trial 3.....	7	6	7	2	3	10	12	3	12	1	2	0
Keratella:												
Trial 1.....	0	1	9	9	9	4	2	0	0	0	16	1
Trial 2.....	1	1	35	11	2	1	23	14	1	0	35	1
Trial 3.....	105	51	105	41	1	1	12	3	12	1	18	2
Asplanchna:												
Trial 1.....	8	10	18	14	18	22	8	3	8	0	7	0
Trial 2.....	12	7	14	13	8	9	98	48	12	0	1	0
Trial 3.....	11	9	10	12	13	21	1	0	1	2	7	2
Brachionus:												
Trial 1.....	122	136	296	252	296	278	144	102	122	10	80	6
Trial 2.....	58	68	72	85	144	148	351	191	58	11	79	24
Trial 3.....	27	24	27	18	281	363	94	88	88	23	84	11
Daphnia:												
Trial 1.....	24	21	10	8	10	3	11	7	24	1	6	0
Trial 2.....	11	16	1	2	11	10	8	1	11	0	7	0
Trial 3.....	5	5	5	7	36	8	7	0	7	0	28	0
Bosmina:												
Trial 1.....	34	43	75	79	79	13	29	4	34	0	37	8
Trial 2.....	76	93	6	7	29	5	68	2	76	4	16	4
Trial 3.....	19	27	19	17	69	33	88	4	88	4	202	8
Diaptomus:												
Trial 1.....	140	116	25	16	15	3	183	8	140	1	166	0
Trial 2.....	23	27	23	9	183	5	21	0	33	0	23	1
Trial 3.....	52	36	52	3	50	2	122	0	122	0	32	0
Cyclops:												
Trial 1.....	20	12	14	6	5	2	12	0	20	1	28	0
Trial 2.....	4	6	5	6	12	1	58	3	14	0	14	0
Trial 3.....	10	14	10	8	10	0	27	1	27	0	85	0
Nauplii: ¹												
Trial 1.....	53	49	63	40	63	15	46	11	53	2	48	0
Trial 2.....	80	71	48	31	46	26	89	12	80	2	80	1
Trial 3.....	50	58	50	46	59	29	73	19	73	7	152	9

¹ Represents both Diaptomus and Cyclops.

TABLE 11.--Percent survival of several aquatic invertebrates after 24 hours' exposure to toxaphene concentrations

Organism and toxaphene concentration	Percent alive
Hirudinea (2 trials):	
1,000 p.p.b.....	100
Amphipoda:	
<i>Gammarus</i> (14 trials):	
100 p.p.b.....	100
200 p.p.b.....	39
300 p.p.b.....	21
500 p.p.b.....	0
Hydracarina (4 trials):	
1,000 p.p.b.....	100
Ephemeroptera:	
<i>Callibaetis</i> (14 trials):	
150 p.p.b.....	100
300 p.p.b.....	71
400 p.p.b.....	13
500 p.p.b.....	0
Odonata:	
<i>Aeschna</i> (17 trials):	
200 p.p.b.....	100
275 p.p.b.....	84
350 p.p.b.....	40
450 p.p.b.....	0
<i>Lestes</i> (21 trials):	
450 p.p.b.....	100
500 p.p.b.....	81
600 p.p.b.....	33
850 p.p.b.....	0
Hemiptera:	
<i>Notonecta</i> (18 trials):	
275 p.p.b.....	100
300 p.p.b.....	71
400 p.p.b.....	39
600 p.p.b.....	0
<i>Sigara</i> (19 trials):	
50 p.p.b.....	100
75 p.p.b.....	60
100 p.p.b.....	25
150 p.p.b.....	0
Trichoptera:	
<i>Limnephilus</i> (12 trials):	
500 p.p.b.....	100
550 p.p.b.....	49
600 p.p.b.....	20
650 p.p.b.....	0
Coleoptera:	
<i>Haliphus</i> (22 trials):	
10 p.p.b.....	100
40 p.p.b.....	45
50 p.p.b.....	11
75 p.p.b.....	0
<i>Hydroporus</i> (18 trials):	
60 p.p.b.....	100
100 p.p.b.....	63
300 p.p.b.....	35
450 p.p.b.....	0
<i>Dytiscus</i> (larvae) (9 trials):	
15 p.p.b.....	100
50 p.p.b.....	76
60 p.p.b.....	58
75 p.p.b.....	0
<i>Gyrinus</i> (16 trials):	
65 p.p.b.....	100
100 p.p.b.....	78
150 p.p.b.....	60
185 p.p.b.....	0
Gastropoda:	
<i>Lymnaea</i> (4 trials):	
700 p.p.b.....	100

tolerance limit (TL_m) of 0.037 p.p.m. for cladocerans, and Hooper and Grzenda (1957) found *Daphnia magna* to have a TL_m of 1.5 p.p.m. at 55° F.

Tolerance levels (100-percent survival) for the larger invertebrates are listed in decreasing order as follows: Hirudinea, Hydracarina, Gastropoda, Trichoptera, Odonata, Hemiptera, Ephemeroptera, Amphipoda, Coleoptera (table 11). Survival at concentrations between 100-percent survival and 100-percent mortality showed an approximate straight-line relation (table 11). Genera within each group

did not exhibit similar tolerance levels. This was evidenced among members of Odonata, Hemiptera, and Coleoptera. Lowered temperatures produced marked increases in tolerance levels. In *Lestes* tolerance increased approximately 35 percent by lowering the temperature 10 degrees. Hooper and Grzenda (1957) found mortality in fathead minnows increased approximately threefold by raising the temperature from 50° F. to 75° F. Many of the findings are similar to those of Prevost (1960), however comparisons are difficult since he provided no temperature data.

DISCUSSION

Populations of plankton show many large variations throughout the year (Pennak, 1949; and Rawson, 1956). In the present study, the populations of organisms which could best illustrate posttreatment changes were not severely reduced, therefore no obvious effects could definitely be attributed to toxaphene treatment. Extensive fish removal can evidently be accomplished without seriously affecting the plankton, but large reductions in these organisms occur at 100 p.p.b. (Wollitz, 1958; and Hoffman and Olive, 1961). However, they reappear while the water is still toxic to fish (Tanner and Hayes, 1955), and begin repopulating before detoxification will permit fish survival.

No marked reductions were observed among most of the larger invertebrates. Hooper and Fukano (1960) reported bottom fauna to be nearly as abundant in two Michigan lakes after treatment (10 p.p.b.) as before, but Stringer and McMynn (1958) found that Amphipoda was eliminated at 10 p.p.b. and Ephemeroptera at 30 p.p.b. Severe reductions in many of these organisms may be expected at higher concentrations. Odonata, Ephemeroptera, Tendipedidae, and *Chaoborus*, were eliminated with 100 p.p.b. toxaphene (Hooper and Grzenda, 1957; and Cushing and Olive, 1957). Unionidae, Sphaeridae, Gastropoda, Oligochaeta, and Hirudinea appear to be more resistant (Hooper and Grzenda, 1957); and Stringer and McMynn, 1958).

Field observations were supplemented by controlled experiments, since most organisms

tested were not reduced at concentrations used for fish removal. It should be recognized that lower tolerance levels probably exist under field conditions which involve longer exposure periods.

SUMMARY

Effects of different toxaphene concentrations on plankton and other aquatic invertebrates were studied under natural and controlled conditions. Five North Dakota lakes were included in the study, which extended from June through September of 1960 and 1961. Physical and chemical data are presented for each lake.

Polyarthra, Keratella, Asplanchna, Conochiloides, Brachionus, Trichocera, Daphnia, Bosmina, Ceriodaphnia, and Cyclops were the dominant zooplankters. No marked reductions were observed after treatment with 5 to 35 p.p.b., but a marked reduction of many plankters followed the second treatment (90 p.p.b.) in Raleigh Reservoir. The Cyanophyta were the most abundant phytoplankters in all lakes. Aphanizomenon increased in all lakes after treatment, but other phytoplankters exhibited no consistent changes. Chlorophyta, Chrysophyta, and Pyrrophyta contributed little to phytoplankton abundance.

The most abundant plant-inhabiting organisms and bottom fauna exhibited no marked changes after treatment. Gammarus, Physa, Gyraulus remained almost constant, while Callibaetis, Caenis, Ischnura, and Tendipes decreased slightly but were again numerous 1 year after treatment.

Tests on several species of zooplankters showed Rotifera to be the most tolerant, followed by Cladocera and Copepoda. Reductions were in Rotifera at 500 p.p.b., in Cladocera at 250 p.p.b., and in Copepoda at 100 p.p.b. Experiments with the larger invertebrates showed Hirudinea, Hydracarina, and Gastropoda to be the most resistant to toxaphene, followed in order by Trichoptera, Odonata, Hemiptera, Ephemeroptera, Amphipoda, and Coleoptera. Survival among the larger invertebrates at intermediate concentrations between 100-percent survival and 100-percent mortality re-

vealed an approximate straight-line relation. Genera within each group exhibited dissimilar tolerance levels.

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INVESTIGATIONS IN FISH CONTROL

**5. Growth Rates of Yellow Perch
in Two North Dakota Lakes
After Population Reduction with Toxaphene**

By Donald C. Warnick, Fishery Biologist



U.S. DEPARTMENT OF THE INTERIOR
Fish and Wildlife Service
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GROWTH RATES OF YELLOW PERCH IN TWO NORTH DAKOTA LAKES AFTER POPULATION REDUCTION WITH TOXAPHENE

By Donald C. Warnick, Fishery Biologist

Abstract.--Growth rates of yellow perch that survived a toxaphene treatment in Brush and Long Lakes in North Dakota were calculated by the scale method for the 1960 and 1961 growing seasons. Brush Lake fish exhibited greatly increased growth rates for both growing seasons following the treatment. Increased growth rates were not evident for Long Lake fish until the 1961 growing season. At the end of the first full growing season after treatment the surviving yellow perch exceeded what may be considered to be the minimum harvestable size of 7 inches. The approximate concentration of toxaphene for reducing the density of fish populations is believed to be 25 percent of the rate determined for fish eradication in most North Dakota waters.

Waters overpopulated with desirable species generally produce few harvestable fish, because of slow growth. Bennett (1962) stated that no fish of harvestable size were found in some waters thus affected; Eschmeyer (1936) made a similar observation concerning overcrowded populations of yellow perch (Perca flavescens). For lack of more efficient remedial measures the use of piscicides has been recommended to reduce the numbers of the problem species.

Relatively low concentrations of toxaphene (chlorinated camphene) in two North Dakota lakes substantially reduced the density of the yellow perch populations; the effect on other fish species was less obvious. The results reported (Henegar, 1965) were incidental to the determination of the minimum toxaphene concentration necessary for fish control in that State. My study was started in 1960 to determine the growth rates of the yellow perch sur-

living in Brush and Long Lakes, and thus gain information concerning the suitability of toxaphene for reducing the numbers of fish in overpopulated waters. The scale method was employed to calculate growth rates of Brush Lake fish for the 1960 and 1961 growing seasons. Posttreatment growth rates of Long Lake fish were determined for part of the 1960 growing season and for all of the 1961 season.

Several authors reporting on the use of rotenone to thin overcrowded populations or to restore balance among fish species considered the results favorable. Beckman (1941) noted that the growth rates of fish surviving the treatment of half of Booth Lake, Mich., were too great to be accounted for by normal variation. Substantially increased harvests, apparently the results of accelerated growth rates of remaining fishes, were reported by Swingle, Prather, and Lawrence (1953) following treatment of some Alabama ponds. Hooper and Crance (1960) stated that the use of rotenone was an effective and economical way to restore balance in certain fish populations. The use of toxaphene was recommended by several authors including Hemphill (1954) who first used the chemical for fish eradication.

This publication is based on a thesis submitted to the Graduate Faculty, Department of Entomology-Zoology, South Dakota State College of Agriculture and Mechanic Arts, in partial fulfillment of the requirements for the degree of Master of Science, June 1963.

THE STUDY LAKES

Cost of fish eradication with toxaphene is approximately 15 percent of the cost with rotenone. With recommended concentrations and methods for thinning overcrowded fish populations with these chemicals, toxaphene is even more economical. Definite information about this use of the poison and the subsequent results is conspicuously absent.

Unfavorable results from the early use of toxaphene for fish eradication were not uncommon and tended to delay the acceptance of the piscicide for use in fishery management (Prevost, 1960). Consequences of a serious nature were the failure of the poison to kill all fish; the extended toxicity of some treated waters; and the reduction or elimination of many aquatic organisms. Hooper and Grzenda (1955) first suggested that such results were due to confusion concerning lethal concentrations and the belief was substantiated by the accumulation of additional evidence (Stringer and McMynn, 1960). Increased proficiency in using the chemical for fish eradication led to its acceptance for that purpose as indicated by Gebhards' 1960 review of past and proposed use in western states.

Toxaphene concentrations used for fish eradication reportedly reduce or eliminate many fish-food and food-chain species, some of which do not reappear in quantity for extended periods (Stringer and McMynn, 1958). Relatively little is known concerning the effects of the lesser toxaphene application rates recommended for reducing the density of overcrowded fish populations. A paucity of fish-food organisms in the North Dakota lakes--even for a comparatively short period after toxaphene application--would affect growth rates of the surviving fish as indicated by scale analysis.

I am obliged to Dale L. Henegar, Chief of Fisheries, North Dakota Game and Fish Department, who brought to my attention the opportunity for this investigation, and to the fishery personnel who assisted with the field work. I wish to thank Marvin O. Allum, Associate Professor of Zoology, for his counsel during the study, and the other faculty members and fellow graduate students of South Dakota State College for their interest and assistance.

The minimum concentration of toxaphene required for fish eradication is determined on the basis of the physical and chemical characteristics of the water for which treatment is proposed in addition to certain biological conditions, and this is assumed to be true with regard to application rates for reducing the density of fish in overpopulated waters. Some physical and chemical characteristics of Brush Lake and Long Lake are presented in table 1. The following history is pertinent to the study of the posttreatment growth rates and is based on material presented by Henegar in 1961 (Henegar, 1966).

Both lakes were treated with toxaphene to produce concentrations of approximately 0.010 parts per million (p.p.m.) using an emulsifiable concentrate containing 6 pounds of the active ingredient per gallon. Brush Lake was treated on October 5, 1959, and Long Lake on July 17, 1960. The method of application was that commonly used by the North Dakota Game and Fish Department, and similar to that described by Stringer and McMynn (1958).

Dilution of the waters by rainfall or by runoff was inconsequential after toxaphene treatment, because of unusual drought. Water levels receded somewhat during the course of the study. Rooted aquatic vegetation was along portions of the shoreline and in several small shallow areas of Long Lake at the time of treatment, but was nearly absent from Brush Lake because of the late-season treatment date.

Apparently, all young-of-the-year yellow perch were eliminated from both lakes.

TABLE 1.--Physical and chemical characteristics of the lakes

Item	Brush Lake	Long Lake
Location (North Dakota).....	McLean County	Bottineau County
General area of State.....	Central	North Central
Origin of lakes.....	Glacial	Glacial
Bottom type.....	Silt-loam	Silt-loam
Surface acres.....	160	291
Acre feet.....	1,527	2,391
Maximum depth (feet).....	23-24	23-24
Average depth (feet).....	9.5	8.2
pH ¹	8.5	8.3
Phenolphthalein alkalinity (p.p.m.) ¹	40	40
Methyl orange alkalinity (p.p.m.) ¹	460	220
Hardness (p.p.m.) ¹	476	308
Total dissolved solids (p.p.m.) ¹	290	307

¹ Condition on date of application.

Observations established deaths of some young-of-the-year northern pike (Esox lucius) in Long Lake, but posttreatment netting disclosed they were not eliminated. The effect of the poison on adult fish of several species in both lakes was less evident than the effect on yellow perch, the dominant species.

Excluding the young-of-the-year, yellow perch density was reduced approximately 91 percent in Brush Lake and 79 percent in Long Lake. The figures are derived from the results of test-netting just before and several months after poisoning. Netting results also indicated that a greater percentage of the smaller yellow perch (less than 140 millimeters) was eliminated than of the larger fish. Observation of Long Lake for several days after toxaphene application tended to substantiate the netting results.

Populations of fathead minnows (Pimephales promelas) were established in the lakes after treatment. Brush Lake was stocked on May 27, 1960, and Long Lake on August 18, 1960. Introduction of minnows after toxaphene treatment is a general practice of the North Dakota Game and Fish Department.

AGE AND GROWTH

Varied evidence has been presented in support of the validity of the scale method for the determination of the age and growth of fishes (Lee, 1920; Van Oosten, 1929). Similar evidence indicates that the method is valid for determining the age and growth rates of yellow perch. Joeris (1957) indicated that additional evidence on the validity of the annulus would accumulate from the further study of Green Bay (Lake Michigan) perch. Jobes, as early as 1934, assumed the validity of the method for yellow perch. The North Dakota study was based on the assumption that the method is valid.

Scale samples of yellow perch from the study lakes were obtained from specimens netted before and after the poisoning, from poisoned fish, and from winterkilled specimens.

It was apparent during analysis of the scale samples from Long Lake, July 12-17, 1960, that

distinction of age classes would be difficult. Determination of the age composition for this group was dependent on the identification of all annuli for each scale sample. For many samples it could not be established whether the 1960 annulus had been formed. The samples from smaller fish (80-120 millimeters) generally evidenced annulus formation and some subsequent growth, but the 1960 annulus was apparently unformed on some of the scales of larger fish. Because of relatively little scale growth the previous season, it could not be determined whether the annulus was recently formed and the later scale growth was of the 1960 growing season, or the annulus was unformed and the scale growth of the previous year was represented. An error of 1 year would be introduced by the wrong choice.

A similar difficulty was noted by Joeris (1957) during analysis of yellow perch scales. Beckman (1943) reported that the time of annulus formation may vary notably among species and within age groups of the same species. Annulus formation probably would have occurred before the July collection date with more favorable growth conditions.

Even without this difficulty, determination of age classes would have been somewhat subjective. Annuli were not distinct, and markings assumed to be false annuli were common. Consequently the age classes and specific growth rates of fish before treatment are not included.

Posttreatment scale samples from both lakes were obtained after the interruption of growth for the 1961 season and before 1962 growth was begun. An annulus was assumed at the scale margin although none was evident. All discernible increase in scale growth of the Long Lake fish was included between the margin and the annulus of the previous year. The scale growth of Long Lake fish during the 1960 season after poisoning was not distinguishable from previous scale growth. Growth increments for the 1961 growing season are presented in table 2. Errors other than mechanical are unlikely because of the distinctive scale growth and the absence of false annuli during that period.

TABLE 2.--Calculated growth increments of yellow perch from Long Lake for the 1961 growing season

[In millimeters]

Number of fish	Total length at capture		Calculated growth increment	
	Range	Average	Range	Average
17 ¹	103-132	115	--	--
1.....	--	178	--	82
5.....	201-210	205	58-101	75
1.....	--	219	--	59
21.....	221-230	227	59-120	74
32.....	231-240	235	45-116	75
28.....	241-250	247	56-106	79
12.....	251-260	253	75-116	83
7.....	261-270	265	54-99	78

¹ Young-of-the-year in 1961.

Scale samples were not obtained from Brush Lake fish until 2 years after treatment. Accelerated scale growth was obvious between the scale margin and annuli of the 2 previous years. The calculated growth rates for the corresponding periods are contained in table 3. As in the scales of Long Lake fish, growth before poisoning was obscured by the presence of numerous false annuli.

When an annulus of the year previous to those located for the preparation of tables 2 and 3 was obvious, as it was on some scales, a direct comparison of scale growth before and after poisoning was made. On this basis the post-treatment growth during the first year was approximately six times greater than for the previous year.

Table 4 shows the relation of fish length to subsequent growth--both calculated. The fact that greater length increments were recorded for smaller fish lends validity to the scale method as applied here.

A change in the size composition of Long Lake fish is evident in table 5. The numbers of fish in the last column represent a subsample of winterkilled specimens in addition to several obtained by qualitative test-netting. The numbers of fish in the other columns represent test-netting results at the times indicated. Excluding the 17 young-of-the-year of 1961, the lengths of fish listed in the last column are approximately 75 to 100 millimeters greater than the lengths of fish listed in the previous column. Despite the time interval, only one growing season, 1961, is represented. The calculated average growth increment for the period was 82 millimeters.

TABLE 3.--Calculated growth increments of yellow perch from Brush Lake for the 1960 and 1961 growing seasons

[In millimeters]

Number of fish	Total length at capture	Average 1960 increment	Average 1961 increment
6 ¹	175-200	95	98
0.....	201-225	--	--
7.....	226-250	89	45
15.....	251-275	95	51
8.....	276-300	100	57

¹ Young-of-the-year in 1960.

TABLE 4.--Relation of fish lengths to subsequent growth increments (both calculated) for yellow perch from Long Lake

[In millimeters]

Number of fish	Calculated length, May 1961	Growth increment 1961	
		Range	Average
17 ¹	103-132	103-132	115
2.....	91-100	82-121	101
2.....	101-110	101-119	110
1.....	111-120	--	--
0.....	121-130	--	--
8.....	131-140	64-116	92
15.....	141-150	61-104	85
27.....	151-160	58-96	76
29.....	161-170	59-101	76
12.....	171-180	67-89	75
8.....	181-190	45-82	67
1.....	191-200	--	66
1.....	201-210	--	54

¹ Young-of-the-year in 1961; lengths are measured total lengths.

TABLE 5.--Toxaphene-effected change in the yellow perch population of Long Lake based on measured total lengths of fish taken during the study

Length range	Number of fish		
	Before treatment (July 1960)	After treatment	
		October 1960	May 1962
45-65 mm.....	1,010	0	0
66-100 mm.....	527	0	0
101-125 mm.....	312	0	1 ¹ 17
126-150 mm.....	198	152	0
151-175 mm.....	196	243	0
176-200 mm.....	7	0	1
201-225 mm.....	0	0	12
226-250 mm.....	0	0	75
251-275 mm.....	0	0	19
Total.....	2,250	395	124

¹ Young-of-the-year in 1961.

DISCUSSION

The yellow perch is a popular species in recreational fisheries, especially for winter fishing, but fish shorter than a total length of 7 inches or approximately 175 millimeters are not often sought and removed by fishermen. If this length is considered the minimum harvestable size, the growth rates recorded for the yellow perch from the North Dakota lakes are significant with respect to the short time required for the improvement of recreational fisheries. All yellow perch surviving similar treatment rates could be expected to exceed the minimum desirable size during the subsequent growing season, and young-of-the-year after only two growing seasons.

Young-of-the-year yellow perch commonly reach harvestable size in three or more growing seasons except in overpopulated waters where growth is restricted. Growth rates of surviving fish were exceptionally rapid during the growing season following treatment--1960 for Brush Lake and 1961 for Long Lake--when compared with growth rates of yellow perch in other areas. Similar growth increments have not been recorded even for more southern latitudes with longer growing seasons (Carlander, 1953).

On the basis of this study, better recreational fishing can be provided at low cost in some lakes and small impoundments overpopulated with yellow perch. Improved angling was assumed in the North Dakota lakes since more fish of harvestable size were produced, but the determination of increased harvests would be conclusive. Comparable results might also be expected following the thinning of other commonly overpopulated species inasmuch as my study was opportunistic, not a deliberate selection of species.

Low toxaphene concentrations in North Dakota lakes have been observed to eliminate small fish of many species including the bullhead (*Ictalurus melas*), reported by Kallman, Cope, and Navarre (1962) to be somewhat resistant to the toxicant. The policy in North Dakota of introducing minnows in waters after treatment with toxaphene is based on this observation.

An assumed absence of prey fish may explain the continued slow growth of yellow perch in Long Lake during the 1960 growing season, after the mid-July application of toxaphene. The May 27, 1960, introduction of 180,000 fathead minnows in Brush Lake evidently assured the presence of significant numbers for the same growing season and no unusual period of slow growth was apparent from scale analysis. The reduction or elimination of prey fish by the mid-July poisoning and the August 18, 1960, stocking of 200,000 fathead minnows, leaves doubt whether significant numbers were present in Long Lake until the 1961 growing season when growth rates of yellow perch were greatly accelerated. A need for more conclusive information concerning the relation of prey species to growth rates is indicated.

Assuming need for the introduction of prey species, a definite advantage is apparent for the fall treatment of waters since stocking can be accomplished early in the subsequent growing season. Treatment in April or May is probably more advantageous than during the growing season, but conditions then are not favorable for rapid detoxification, and stocking of prey species might have to be delayed.

The toxaphene treatment rate which allowed the survival of yellow perch in the North Dakota lakes was approximately one-third of the determined rate for fish eradication in most waters of that State. A belief that the reductions were excessive can be temporized since the possibility that an optimum number of fish survived in either lake is unlikely, and greater growth rates could hardly be expected. Test-netting of both lakes and observation after a partial winterkill of Long Lake in 1962 substantiates the belief. Concentrations approximating 0.008 p.p.m. (one-fourth of the minimum lethal rate) probably would have allowed the survival of more fish without significantly reducing growth rate.

On the basis of a recent report by Kallman, Cope, and Navarre (1962), the presence of relatively large quantities of vegetation at the time of treatment could affect the outcome, especially in consideration of the low toxaphene concentrations required for the thinning of fish populations. It was indicated that high concentrations of toxaphene are accumulated by certain vegetative species in a relatively short time, thus essentially removing the chemical from the water--at least temporarily--and the further disposition of the chemical was unknown. Therefore, more consistent results might be obtained, with regard to the degree of reduction, by treatment during the absence of most aquatic vegetation.

The appropriate reduction for any population necessarily depends on a variety of conditions, some unknown. The difficulty of determining the magnitude of fish populations, particularly after treatment, seriously affects an evaluation of the results. Additional information concerning the use of toxaphene for reducing the density of fish populations is needed for its greatest usefulness.

SUMMARY AND CONCLUSIONS

Yellow perch populations in two North Dakota lakes were substantially reduced by low toxaphene concentrations. Growth rates of surviving fish were determined by the scale method for 2 years after treatment. Greatly increased growth rates were evident for both growing seasons following the fall treatment of Brush Lake. Increased growth rates were not evident for Long Lake fish after the July 17, 1960, treatment until the 1961 growing season. All yellow perch surviving the poisoning exceeded what may be considered to be the minimum harvestable size during the first full growing season after treatment. Comparable results can be expected from similar and perhaps lesser reductions with toxaphene.

Further use of toxaphene is recommended for reducing the density of yellow perch populations and thus improving certain recreational fisheries. Other species might be similarly managed. Reduction or elimination of prey species was believed to explain the continued slow growth of Long Lake fish for approximately 2 months after toxaphene application. Fall treatment is apparently the most timely, especially with regard to assuring the presence of significant numbers of prey species during the growing season. Numerous conditions affect the concentration of toxaphene needed for fish eradication, and the approximate concentration for reducing the density of fish populations is believed to be 25 percent of that rate.

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INVESTIGATIONS IN FISH CONTROL

**6. Mortality of Some Species of Fish
to Toxaphene at Three Temperatures**

By Mahmoud Ahmed Mahdi, Fishery Biologist



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MORTALITY OF SOME SPECIES OF FISH TO TOXAPHENE AT THREE TEMPERATURES

By Mahmoud Ahmed Mahdi, Fishery Biologist

Abstract.--Lethal concentrations of toxaphene were determined for the stoneroller, golden shiner, goldfish, black bullhead, and bluntnose minnow in water at 53° F., 63° F., and 73° F.; rainbow trout were tested at 53° F. The TL_m and LD_{50} were obtained by graphic methods. For comparison a normit method was used with the bluntnose minnow data; all three methods gave similar results for the bluntnose minnow. In most experiments, concentrations of toxaphene needed to cause 50 percent mortality decreased as the temperature increased from 53° F. to 63° F. and to 73° F. The 96-hour TL_m values were below 0.1 p.p.m. of toxaphene in all species tested. Goldfish were the most tolerant, with the other species showing so similar a sensitivity that they could not be effectively ranked by these data.

Toxaphene (chlorinated camphene) is used widely as an insecticide on farm crops and as a poison to control fish populations (Henderson et al., 1959; Fukano and Hooper, 1958). Toxaphene is highly toxic, especially to man, and is never recommended for household use (it can be absorbed through the skin). For mammals, toxaphene is one of the more toxic of the chlorinated hydrocarbon insecticides (Cohen et al., 1960, p. 1552). Negherbon (1959) lists it as being more toxic to fish than DDT or rotenone. Toxaphene is nonvolatile, yellow to amber in color, and has an aromatic, pinelike smell. It is insoluble in water but soluble in organic solvents (Henderson et al., 1959). The emulsion used in this investigation was milky white.

This study was an attempt to determine the concentrations of toxaphene that will kill certain species of fish. Three temperatures were used, to ascertain the role temperature plays in the toxicity of toxaphene.

This publication is based on a thesis submitted to the Graduate Faculty, Iowa State University of Science and Technology, in partial fulfillment of the requirements for the degree of Master of Science. Studies were conducted under a grant made through the Sudanese Government by the Agency for International Development.

I am indebted to Dr. Kenneth D. Carlander for his help in interpreting the data and in putting this work in its final form. I am obliged also to T. Hoage, Dr. V. Gooch, J. Reynolds, and C. Caillouet for their advice on interpretation of the data, and to Charles Walker of the Fish Control Laboratory, La Crosse, Wis., who supervised the experiments.

METHODS

These experiments were carried out at the Fish Control Laboratory of the Bureau of Sport Fisheries and Wildlife at La Crosse, Wis. The fish were provided by the Laboratory and from various sources, including hatcheries. The following species were tested:

1. Rainbow trout, Salmo gairdneri.
2. Stoneroller, Campostoma anomalum.
3. Goldfish, Carassius auratus.
4. Golden shiner, Notemigonus crysoleucas.
5. Bluntnose minnow, Pimephales notatus.
6. Black bullhead, Ictalurus melas.

Before starting an experiment the fish were examined thoroughly to make sure that there were no signs of disease. They were left in

large holding tanks and fed regularly until 2 days before the start of the experiment. To keep to a minimum waste products that might affect toxicity, they were not fed during the experiments.

WATER

Deionized water was reconstituted by adding a mixture of 0.45 grams of calcium sulphate, 0.45 grams of manganous sulphate, 0.72 grams of sodium bicarbonate, and 0.03 grams of potassium chloride, giving 1.65 grams of a powdered mixture for every 15 liters of water. This reconstituted water has the following characteristics:

pH	7.9
Carbon dioxide	1.0 p.p.m.
Total alkalinity	36.0 p.p.m.
Total hardness	72.0 p.p.m.
Calcium hardness	26.0 p.p.m.
Manganese	0.075 p.p.m.
Sulphate ion	37.5 p.p.m.
Chloride ion	6.86 p.p.m.
Ammonia nitrogen	0.05 p.p.m.
Total ion	0.00 p.p.m.

The reconstituted water was aerated for 24 hours and was then transferred into glass jars of two sizes, large ones containing 15 liters of water and small ones of 1 gallon each. Each sized jar was large enough to hold the required volume of water at a point somewhat below the rim. The large jars were placed in concrete tanks about 30 inches deep and 3 feet wide, where they were allowed to rest in the water. Eighteen jars were used in each experiment, including three for controls and three each for five different concentrations. Sometimes additional jars were used to permit more than five concentrations in an experiment. The small jars were put in aluminum troughs 1 foot deep; four small jars were used for the control and 15 for each concentration.

Circulating water pumps were used to mix water in tanks and aluminum troughs to ensure an even temperature. Elevated temperatures were maintained with electric water heaters and thermostats. A small thermograph recorded the temperature of the water, and these records indicated good temperature control.

TRANSFER OF FISH

Hand nets that were used to take fish out of the tanks were kept in an antiseptic solution (38 milliliters of Roccal to 25,000 milliliters of water) when not in use. The fish were transferred into a bucket of water mixed with acriflavine (concentration not critically determined). Douglas and Irwin (1962) found that Terramycin and acriflavine in small amounts were successful in combating fin or tail rot. The fish were not overcrowded, but to ensure an abundance of oxygen, air was run into the bucket. During the transfer, any fish which fell to the floor were not used in the experiment.

After making sure that the water temperature in the jars was adjusted, the fish were weighed and placed in the jars to be acclimatized for another 24 hours. To ensure that the fish were not overcrowded, they were introduced according to the loading capacity of each species by weight, making sure that the weight of fish should not exceed the safe range. In the case of the lowest temperature used, 53° F., the safe range of the loading capacity of each jar for different species of fish had been determined by the staff of the Fish Control Laboratory. Experiments were run to determine the safe loading capacity at 73° F. of several species of fish (table 1). From these data it is clear that up to 2 grams of goldfish per liter of water can be used safely for a period of 96 hours. For the golden shiner and the stoneroller, up to 1 gram of fish per liter of water can safely be used for 96 hours. Two grams of golden shiner per liter of water can be used, but not for more than 72 hours, whereas the same loading capacity is not advisable for the sonteroller even for the first 24 hours.

The largemouth bass can stand up to 2 grams of fish per liter of water only for the first 24 hours. If bass are to be used for more than 24 hours, one-fourth gram of fish per liter of water could only be used for not more than 48 hours. Otherwise less loading should be tried.

No loading capacity tests were run at 63° F., but loading capacities exceeding those found at 73° F. were not used.

TABLE 1.--Percentage mortality of four species of fish, and dissolved oxygen in parts per million, at different loading capacities at 73° F.

Fish and time	At a load per liter of water of--							
	0.25 gram of fish		0.5 gram of fish		1.0 gram of fish		2.0 grams of fish	
	Fish killed	Oxygen	Fish killed	Oxygen	Fish killed	Oxygen	Fish killed	Oxygen
	Percent	P.p.m.	Percent	P.p.m.	Percent	P.p.m.	Percent	P.p.m.
Stoneroller:								
24 hours.....	0	--	0	--	0	--	38	--
48 hours.....	0	3.5	0	3.5	0	1.6	38	1.6
72 hours.....	0	2.6	0	2.6	0	1.4	38	1.3
96 hours.....	0	2.8	0	2.7	0	1.5	50	1.3
Goldfish:								
24 hours.....	--	--	0	--	0	--	0	--
48 hours.....	--	--	0	2.9	0	1.4	0	0.9
72 hours.....	--	--	0	1.7	0	1.0	0	0.8
96 hours.....	--	--	0	2.0	0	1.6	0	0.8
Golden shiner:								
24 hours.....	0	--	0	--	0	--	0	--
48 hours.....	0	5.3	0	3.8	0	1.9	7	1.6
72 hours.....	0	3.9	0	2.7	0	3.1	7	1.5
96 hours.....	0	3.5	0	3.1	0	3.7	15	1.5
Largemouth bass:								
24 hours.....	0	--	0	--	8	--	4	--
48 hours.....	0	5.3	33	4.3	31	2.2	22	0.9
72 hours.....	25	4.1	33	2.5	31	1.6	26	0.8
96 hours.....	25	3.7	33	2.5	31	2.0	26	1.3

INTRODUCTION OF TOXAPHENE

Toxaphene solution was prepared fresh from commercial stock each time it was to be introduced into the jars. The commercial stock was a solution of 4 pounds per gallon. Two milliliters of the commercial stock were transferred to a 1-liter flask, and deionized water was added to make 1 liter of solution. When 1 milliliter of this solution was added to 1 liter of water it gave approximately 1 part per million of the toxicant. The toxaphene concentration was calculated according to this and introduced into the different jars as required. When very dilute solutions were required, the stock solution was further diluted by taking 100 milliliters of it and adding 1 liter of deionized water, making a new stock solution (0.0001 toxaphene) of which 1 milliliter in 1 liter of water gave 0.1 p.p.m.

In the introduction of the toxicant, 1-milliliter, 2-milliliter, and 5-milliliter pipettes were used. To avoid suction of the toxicant and to speed the introduction of the toxaphene into the jars, the stock solution was put in a long cylinder and the pipette filled by capillary action. When the level of the solution in the cylinder went down, more was added from the original solution. A glass rod was used to ensure mixing of the toxicant. The concentration of each jar was noted by marking with a wax pencil on the logs of wood supporting the big jars or on the aluminum tank near each small jar.

OBSERVATIONS

Observations were recorded each 24 hours after the toxaphene was introduced. Readings were taken for 96 hours, showing the number of dead fish in each jar. Oxygen was tested each day from the day of introduction of toxaphene to the last day of the experiment. Samples for oxygen determination were taken from the controls and not always from the same jar each day. This does not show exactly the oxygen situation in all the jars. There is a greater chance for more oxygen in the jars that lost larger numbers of fish (died and removed) than jars with most of the fish still alive.

Unless a fish was completely dead, it was recorded as alive. Some fish, bluntnose minnows for example, rested at the bottom of the container with their backs down and appeared to be dead. The fish in such cases were touched with a glass rod; if they did not show signs of movement, they were recorded as dead. Dead fish were removed only once a day. Each day the dead fish were collected and burned. At the end of the experiment all treated fish, including those still alive, were disposed of in the same way.

ANALYSIS

From the mortality observed, the TL_m (median tolerance limit) values were obtained. The TL_m is "the concentration of the tested

material in a suitable diluent (experimental water) at which just 50 percent of the test animals are able to survive for a specified period of exposure" (American Public Health Association, 1960, p. 458).

On semilogarithmic paper, 3 cycles x 70 divisions, concentration of the toxicant was plotted against the mortality observed. Two points only were used, one point showing mortality above 50 percent of the population and the other one below 50 percent. These two points were connected (see fig. 1). From this line the concentration which killed 50 percent of the population was obtained. In this example the 96-hour TL_m for bluntnose minnows at 63° F. was found to be 0.0088 p.p.m. In certain cases where the gap between the two points was big, for instance, goldfish at 63° F., the 72-hour TL_m values were obtained but were considered rough.

Whenever there were sufficient data, the LD_{50} (the median lethal dose) was evaluated by a graphic method (Wilcoxon and Litchfield, 1949). The does were plotted against the percent mortality on logarithmic-probability paper No. 3228. Tests showing 0 percent or 100 percent mortality were omitted. A straight line was drawn through the points, particularly those

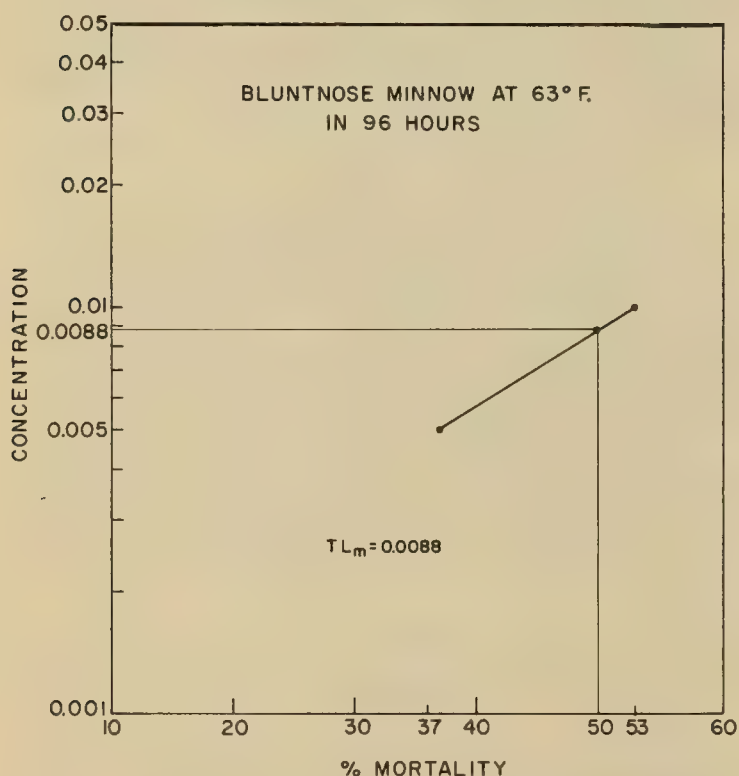


Figure 1.--The 96-hour TL_m for bluntnose minnows at 63° F.

showing just less and just more than 50 percent mortality (fig. 2). The expected mortality at each concentration is then read from the straight line and compared with the observed mortality (table 2). These authors stated that if the chi square of the line is less than the value of chi square given in table 2 for degrees of freedom, the data are not significantly heterogeneous, that is, the line is a good fit.

Since 0.0502 is less than 7.82, the line seems to be a good fit. From this line (fig. 2) the concentration of the toxicant which kills 50 percent of the population was read and the LD_{50} was found to be 0.011 p.p.m.

In cases where there were only two points (above and below 50 percent mortality), LD_{50} values were determined but they could not be checked statistically as the number of degrees of freedom equals 0.

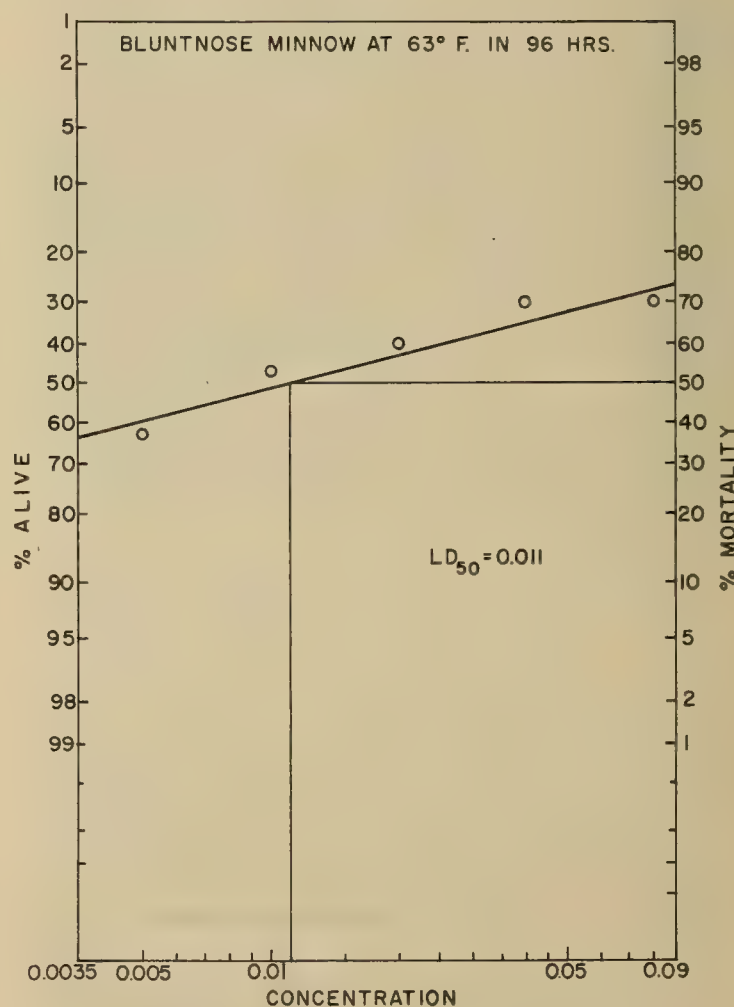


Figure 2.--The 96-hour LD_{50} for bluntnose minnows at 63° F.

TABLE 2.--Chi-square test of the 96-hour LD₅₀ for bluntnose minnows at 63° F.

Toxaphene concentration	Percentage mortality		Difference	Contribution to chi square
	Observed	Expected		
0.005 p.p.m.....	37	40	3	0.0036
0.01 p.p.m.....	53	59	4	0.0060
0.02 p.p.m.....	60	57	3	0.0035
0.04 p.p.m.....	70	65	5	0.0100
0.08 p.p.m.....	70	72	2	0.0020
All.....	--	--	--	0.0251

For the bluntnose minnows a third method of analysis was also used, the minimum normit chi square method (Berkson, 1955). The LD₅₀ is 0.0092 p.p.m. which is quite close to the TL_m and graphic LD₅₀ values.

RESULTS

The three methods of analyzing the data gave very similar results in most cases when applied to the bluntnose minnow (table 3). At 24 hours at 53° F, none of the concentrations gave over 22 percent mortality, and therefore the TL_m and LD₅₀ values must be very approximate. At 63° F, the mortality was spread over a wide range of concentrations with lower mortality sometimes appearing in higher concentrations. With such variation in the experimental data it can be expected that the estimates of the concentration at which 50 percent die is subject to considerable variation. Yet the three methods give quite comparable estimates except for the 48-hour period. At 73° F, the 48-hour TL_m and normit LD₅₀ values do not agree very well. This results from the use of only two points in each case, and the two points used were not the same.

The normit method was used only with the bluntnose minnow. In several situations with

TABLE 3.--TL_m and LD₅₀ values for bluntnose minnows at three temperatures.

Temperature and time	TL _m	LD ₅₀	
		Graphic	Normit
At 53° F:			
24 hours.....	>4.0	--	11.58
48 hours.....	0.86	0.86	0.93
72 hours.....	0.15	--	0.118
96 hours.....	0.03	0.03	0.038
At 63° F:			
24 hours.....	0.2	0.15	0.1849
48 hours.....	0.035	0.088	0.04609
72 hours.....	0.014	0.016	0.01805
96 hours.....	0.0088	0.011	0.0092
At 73° F:			
24 hours.....	0.015	0.016	0.0124
48 hours.....	0.0084	--	0.0043
72 hours.....	0.0065	0.0088	0.0071
96 hours.....	0.0063	0.007	0.0066

other species the graphic LD₅₀ method could not be used because mortality rates both above and below 50 percent, but not 0 or 100 percent, were not available. In all other cases the TL_m and LD₅₀ values were quite similar.

Berkson (1950) used eight different methods for the estimation of the LD₅₀ and found that all gave about the same results. Since the three methods used here gave close results in most cases, only the TL_m data are discussed (table 4).

CHANGE WITH TIME

It can be anticipated that fish can survive higher concentrations of a toxicant for short periods of time than they can for longer periods. As expected, in most of the tests (table 4) the TL_m values decreased with time; in some cases the TL_m remained constant, for instance the 72-hour and 96-hour TL_m of the golden shiner at 73° F. and of the goldfish at 53° F. and 63° F., and the 48-hour, 72-hour, and 96-hour TL_m of the goldfish at 73° F. Henderson and Tarzwell (1957) stated that experience with a variety of industrial effluents has shown major difference in 24- and 48-hour TL_m values, although there was little or no difference in the 48- and 96-hour values, except for an occasional effluent which produced fish mortality over the longer period.

Pickering et al (1962) when examining the acute and chronic or accumulative toxicity of Delnav to fathead minnows, found that TL_m

TABLE 4.--Median tolerance limits (TL_m) to toxaphene for six species of fishes at three temperatures.

Species and temperature	Concentration in p.p.m. for--			
	24 hrs.	48 hrs.	72 hrs.	96 hrs.
Rainbow trout:				
At 53° F.....	0.03	0.0145	0.0111	0.0084
Stonerollers:				
At 53° F.....	0.062	0.027	0.027	0.014
Do.....	0.028	0.0086	0.008	0.0066
At 63° F.....	0.054	0.044	0.035	0.032
At 73° F.....	0.009	0.0078	<0.005	<0.005
Goldfish:				
At 53° F.....	0.27	0.115	0.094	0.094
At 63° F.....	0.086	0.035	0.028	0.028
At 73° F.....	0.054	0.05	0.05	0.05
Golden shiner:				
At 53° F.....	0.0125	--	--	--
At 63° F.....	0.027	0.007	0.0062	<0.005
At 73° F.....	0.0134	0.0066	0.006	0.006
Bluntnose minnow:				
At 53° F.....	>4.0	0.86	0.15	0.03
At 63° F.....	0.2	0.035	0.014	0.0088
At 73° F.....	0.015	0.0084	0.0065	0.0063
Black bullhead:				
At 53° F.....	>0.048	>0.048	0.034	0.025
At 63° F.....	0.015	0.0043	0.0042	0.0027
At 73° F.....	0.012	0.0042	0.003	0.0018

values decreased (toxicity increased) rapidly for the first 5 days and less rapidly for the next 10 days. They did not notice any further decreases in TL_m values during the additional 15 days of exposure.

CHANGE WITH TEMPERATURE

With increase in temperature the rate of metabolism is higher and the toxicant is expected to be more effective. This was very clear in the case of the black bullhead and the bluntnose minnow which showed rapid decrease in TL_m with increase in temperature. The goldfish showed rapid decrease in TL_m with increase in temperature from 53° F. to 63° F. At 73° F. the 24-hour TL_m went down and then kept constant at the 48-hour, 72-hour, and 96-hour TL_m values higher than those at 63° F.

The golden shiner and the stoneroller showed decrease in TL_m with change in temperature from 63° F. to 73° F. When temperature was changed from 53° F. to 63° F., the 24-hour TL_m of the golden shiner was higher at 63° F. than at 53° F. The 24-hour TL_m of the stoneroller at 53° F. was higher in one test when compared with the TL_m at 63° F. and lower in the other. Both experiments of stoneroller at 53° F. were lower in 48-hour, 72-hour, and 96-hour TL_m s than the equivalent TL_m at 63° F. These variations in results indicate tests which should be repeated.

SPECIES COMPARISONS

Toxaphene was highly toxic to fish with 96-hour TL_m values below 0.1 p.p.m. in all the tested cases. Henderson et al. (1959) gave TL_m s of four species of fish to toxaphene (table 5). Their results for the fathead minnow are very similar to ours for bluntnose minnows (table 4). The TL_m values for goldfish are somewhat different, but the TL_m values for

goldfish at 73° F. (table 4) have already been questioned. When they used BHC insecticide at 77° F. they found the 24-, 48-, and 96-hour TL_m for the fathead to be 22, 16, 15 p.p.m. respectively and for the goldfish 26, 21, and 15 p.p.m.

When comparing the action of toxaphene in a period of 24 hours, the bluntnose minnow at 53° F. seemed to be the most tolerant of all the tested species. These fish settle on their backs and remain in a half-dead state thus tolerating high doses of the toxicant. The goldfish ranked second, followed by the stoneroller, black bullhead, rainbow trout, and golden shiner. At 63° F. the black bullhead was more sensitive than the golden shiner, whereas the others ranked the same as at 53° F. (no data on the rainbow trout). At 73° F. the goldfish showed higher TL_m than the bluntnose minnow, followed by the golden shiner, the black bullhead, and the stoneroller.

In comparison of the 96-hour TL_m at 53° F., the goldfish was the most tolerant, followed by the bluntnose minnow, black bullhead, and stoneroller, with the rainbow trout as the most sensitive (no data on the golden shiner). At 63° F. the stoneroller was the most tolerant, leaving the goldfish in the second rank, followed by the bluntnose minnow, black bullhead, and golden shiner. At 73° F. the goldfish appeared to be the most tolerant, followed by the bluntnose minnow, golden shiner, black bullhead, and stoneroller.

In general, it seemed that the goldfish was the most tolerant of the tested fish to toxaphene, realizing that the bluntnose minnow at 53° F. showed higher TL_m in the first 72 hours.

This apparent high tolerance of the bluntnose minnow in the first 3 days was due to the fact that they remained on their backs, but still half alive, for some time after being affected. If the half-dead fish had been considered dead, the TL_m values would probably have been lower than those of the goldfish, which died quicker. The other species seem to be about equally sensitive to toxaphene and cannot be readily ranked.

TABLE 5.--Median tolerance limits (TL_m) to toxaphene at 77° F. for four species of fish.

[From Henderson et al., 1959, p. 27]

Fish	Toxaphene, reference standard, 100-percent active in acetone, diluted in--	TL_m (p.p.m.) at--		
		24 hrs.	48 hrs.	96 hrs.
Fatheads..	Soft water.....	0.013	0.0075	0.0075
Do.....	Hard water.....	.016	.0075	.0051
Bluegills.	Soft water.....	.0075	.0038	.0035
Goldfish..do.....	.0082	.0068	.0056
Guppies...do.....	.042	.024	.020

OXYGEN

Aeration of the water was not consistent enough for the experiments to be comparable with respect to dissolved oxygen. Dissolved oxygen recorded at the beginning of the experiments (24 hours after the fish were put in the jars) varied from 5.9 to 8.0 p.p.m. at the 53° F., from 4.8 to 6.2 at 63° F., and from 3.2 to 6.0 p.p.m. at 73° F. More consistent results might have been secured if all experiments had been started with dissolved oxygen near saturation.

In four experiments (stoneroller at 73° F., bullhead at 73° F., and bluntnose minnow at 53° F. and at 73° F.), the recorded dissolved oxygen increased at least 1 p.p.m. sometime during the experiment, a situation which is difficult to explain.

The dissolved oxygen was below 3 p.p.m. at the end of the first 24 hours in the goldfish experiments at 53° F. and 73° F., the two experiments with the highest loading capacity, 2 grams per liter. It was also below this level for the stoneroller at 63° F. and for all 73° F. experiments except the bluntnose minnow. While minimal oxygen requirements of fish are subject to many factors such as temperature and carbon dioxide content, the dissolved oxygen, if at the recorded values, was probably low enough to seriously affect the experimental fish. The Aquatic Life Advisory Committee of the Ohio River Valley Water Sanitation Commission states (1955, p. 327):

The dissolved oxygen content of warm water fish habitats shall be not less than 5 p.p.m. during at least 16 hr. of any 24-hour period. It may be less than 5 p.p.m. for a period not to exceed 8 hr. within any 24-hr. period, but at no time shall the oxygen content be less than 3 p.p.m. To sustain a coarse fish population the dissolved oxygen concentration may be less than 5 p.p.m. for a period of not more than 8 hr. out of any 24-hr. period, but at no time shall the concentration be below 2 p.p.m.

Rounsefell and Everhart (1953) mentioned that the 5 p.p.m. oxygen tolerance limit is too high and listed minimum oxygen requirements as low as 0.38 p.p.m. for largemouth bass, 0.2-0.3 p.p.m. for black bullhead, and below 0.2 p.p.m. for golden shiner.

The fact that no fish died in the controls indicates that oxygen deficiency in itself was not a direct cause of mortality.

SUMMARY

Experiments were run at the Fish Control Laboratory, Bureau of Sport Fishery and Wildlife, La Crosse, Wis., in the summer of 1962, to determine the mortality of the stoneroller, golden shiner, goldfish, black bullhead, and bluntnose minnow to toxaphene in water at 53° F., 63° F., and 73° F. A sixth species, rainbow trout, was tested at 53° F. only.

At the start of testing, all fish were in good health and showed no disease or abnormalities; fish were not fed during the experiments. Deionized water was reconstituted to give a standard water with specified characteristics. Air was run in the reconstituted water for 24 hours before the start of an experiment. A reliable temperature control device was used to ensure constant temperature. Fish were introduced into jars to acclimatize for 24 hours before treatment with various concentrations of toxaphene. Mortality of fish was recorded every 24 hours for 4 days. Untreated controls were used in each experiment.

TL_m and LD₅₀ were obtained by graphic methods to get the concentrations which will kill 50 percent of the population. A normit method was used with the bluntnose minnow data only for comparison. The three methods gave similar results. In most experiments, concentrations of toxaphene needed to cause 50 percent mortality decreased as the length of exposure increased from 24 to 96 hours, and decreased as temperature increased from 53° F. to 63° F. and to 73° F. Dissolved oxygen did not show consistent results and was below 3 p.p.m. in several tests. At 96 hours the TL_m values were below 0.1 p.p.m. of toxaphene in all species, indicating that fish are quite sensitive to toxaphene.

In general, goldfish was the most tolerant of the species tested with the other species showing similar enough sensitivity that they cannot

be effectively ranked with these data. Bluntnose minnows remained half dead much longer than other species, and thus the TL_m values for the first 3 days at least may be somewhat high. Running experiments for longer periods of time is suggested. Narrower ranges of concentrations with several intermediate concentrations can be used in further tests now that the general range has been determined.

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INVESTIGATIONS IN FISH CONTROL

**7. Treatment of East Bay, Alger County, Michigan
with Toxaphene for Control of Sea Lampreys**

By William E. Gaylord, Biological Field Station, Ludington, Mich.
and Bernard R. Smith, Biological Field Station, Marquette, Mich.
Bureau of Commercial Fisheries



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Fish and Wildlife Service

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FOREWORD

Application of toxaphene to East Bay had the approval of the Michigan Department of Conservation and of local inhabitants who were advised on the project in public hearings. The Bureau of Commercial Fisheries and the Great Lakes Fishery Commission, after this single experiment, abandoned the use of toxaphene in the program for control of the sea lamprey. It is believed that certain of the observations in East Bay are of sufficient interest to warrant publication of this report. This study was part of a program conducted by the Bureau of Commercial Fisheries under contract with the Great Lakes Fishery Commission.

TREATMENT OF EAST BAY, ALGER COUNTY, MICHIGAN, WITH TOXAPHENE FOR CONTROL OF SEA LAMPREYS

By William E. Gaylord, Biological Field Station, Ludington, Mich.
and Bernard R. Smith, Biological Field Station, Marquette, Mich.
Bureau of Commercial Fisheries

Abstract.--An experiment was conducted to determine whether toxaphene can be used to eradicate lake-dwelling sea lampreys and to determine its effect on fish populations. In East Bay, a 78-acre lake on the Sucker River, Alger County, Mich., an estimated concentration of 100 parts per billion was maintained for 14 days. The sea lamprey larvae were more resistant to toxaphene than were the fish, but a complete kill was indicated. One year after treatment, sea lampreys were absent from the lake, while the fish population had recovered.

The Bureau of Commercial Fisheries' program for control of the sea lamprey (Petromyzon marinus) in the Great Lakes has progressed rapidly with the development of lamprey larvicides (Applegate et al., 1961). Success of chemical control depends on treatment of all populations of ammocetes in the streams and lakes tributary to the Great Lakes.

The selective lampricide now being used has proved successful in streams and rivers, but its relatively high cost prohibits use in the larger estuarine bodies and lakes in river systems. In many of these a general toxicant can be used without permanent damage to fish populations. Several chemicals are readily available at nominal cost.

Toxaphene (chlorinated camphene) has been used widely as an agricultural insecticide; it has been used to some extent as a fish toxicant, but no reference could be found to its effect on lampreys. Hooper and Grzenda (1957) stated that 0.05 p.p.m. of emulsified toxaphene is sufficient for fish eradication. They also demonstrated that the substrate influences the rate of detoxification of the material. Hooper and Fukano (1960) observed a slowdown in fish

mortality with falling temperatures, particularly at values below 50° F.

An experiment to test the feasibility of using a commercial formulation of toxaphene was planned for 1961. The site selected was East Bay, a small lake in the lower end of the Sucker River, 2 miles east of Grand Marais, Alger County, Mich. The Sucker River had been treated with selective larvicide in 1958 and 1959. The toxaphene formulation used was Cooper-Tox No. 6 (toxaphene emulsifiable concentrate).

STUDY AREA

East Bay is a 78-acre lake formed by low, shifting sand dunes on the shore of Lake Superior (fig. 1). The margin of the lake has a narrow, shallow-water shelf beyond which water depth drops abruptly to 20-30 feet. The bottom is primarily sand; some limited areas have bottoms of soft mud and silt. The Sucker River flows into the southeast end of East Bay. The outlet is on the west side into West Bay, which is connected directly to Lake Superior. The inflow averages approximately 85 c.f.s., but it varied from 80 to 200 c.f.s. during the treatment period.

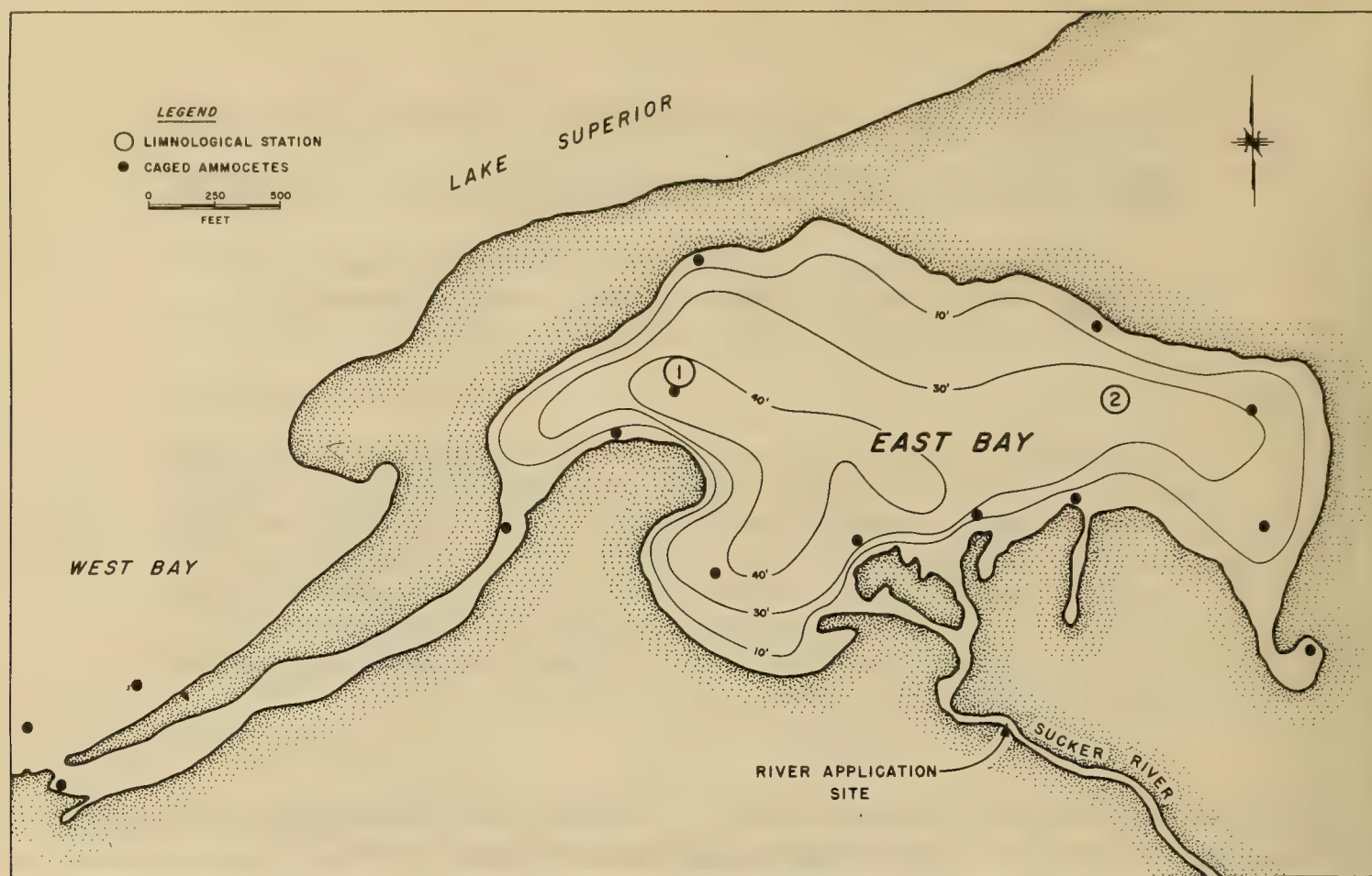


Figure 1.--Map of East Bay showing location of ammocete cages, limnological stations, and the application site in the Sucker River.

East Bay was not stratified thermally in the fall of 1961. Water temperatures varied from a maximum of 49° F. to a minimum of 42° F.; the difference between the surface and the bottom was nil to 2.5° F. (table 1).

Dissolved oxygen remained high (8.9 to 10.2 p.p.m.) during the period of observation. The hydrogen-ion concentration varied from pH 7.5 near the bottom to 9.5 near the surface.

PRETREATMENT SURVEYS

Pretreatment surveys with electric shockers had demonstrated the presence of sea lamprey ammocetes in large numbers in East Bay. The greatest concentrations were on the alluvial fan formed by the Sucker River. The size of the population was estimated at 96,000 individuals (Wagner and Stauffer, 1962). Ammocetes were found also in the connecting channel between

East and West Bays and in West Bay in the immediate area of the mouth of the channel.

A riffle fyke net fished in the channel between East Bay and West Bay from March 15 to October 24, 1961, captured 278 sea lampreys (9 transformed individuals and 269 larvae) and 220 American brook lamprey larvae. These data supported the view that a large population remained in the lake, although the Sucker River had been successfully treated with selective larvicide in 1959.

TABLE 1.--Water temperatures (°F.) in East Bay, October-November 1961

Depth	October 4, at station--		October 25, at station--		November 1, at station--		November 6, at station--	
	1	2	1	2	1	2	1	2
Surface....	49.0	49.4	45.0	45.0	43.0	43.0	42.0	42.0
10 feet..	48.2	48.8	--	--	--	--	--	--
20 feet..	47.2	48.2	--	--	--	--	--	--
30 feet..	46.5	47.0	--	--	--	--	--	--
Bottom ¹	46.5	47.0	45.0	45.0	43.0	43.0	42.0	42.0

¹ 39 feet at station 1, 34 feet at station 2.

Tows, totaling 43 minutes trawling time, with an electric beam trawl at several stations in East Bay in August 1961 captured 52 sea lampreys and 44 American brook lampreys. September 20-22, 151 minutes of trawling in West Bay yielded 16 sea lampreys and 14 brook lamprey larvae. All were captured near the mouth of the channel connecting the two bays.

The fish population was sampled immediately before the treatment by sets of gill nets at 7 locations. Each set included four 125-foot nets with 25-foot sections of 1-1/2-, 2-, 2-1/2-, 3-, and 4-inch mesh (extended measure). The catch in 7 nights of fishing was 27 rainbow trout, 8 northern pike, 41 white suckers, 47 yellow perch (all less than 6 inches long), and a few individuals of 6 other species, to bring the total to 141 fish (table 2).

TABLE 2.--Number of fish, by species, taken from East Bay before and after poisoning with toxaphene

Species	Before treatment (October 5-11, 1961)		After treatment (October 23-25, 1962)	
	Total number	Number per 1,000 feet of net	Total number	Number per 1,000 feet of net
Rainbow trout:				
12 inches and over.....	24	6.9	4	2.7
Less than 12 inches.....	3	0.9	34	22.7
White sucker:				
9 inches and over.....	30	8.6	11	7.3
Less than 9 inches.....	11	3.1	9	6.0
Northern pike.....	8	2.3	2	1.3
Round whitefish.....	6	1.7	1	0.7
Yellow perch.....	47	13.4	3	2.0
Walleye.....	1	0.3	0	0.0
Alewife.....	5	1.4	0	0.0
Burbot.....	2	0.6	0	0.0
Rock bass.....	3	0.9	0	0.0
Brook trout.....	1	0.3	0	0.0
Brown trout.....	0	0.0	1	0.7
Smelt.....	0	0.0	2	1.3
Brown bullhead.....	0	0.0	1	0.7
Total.....	141	40.3	68	45.3

BIOASSAYS WITH TOXAPHENE

Bioassays were conducted, at the Bureau's Hammond Bay Biological Station, of various concentrations of toxaphene to test the effect of the material on lamprey larvae. The laboratory tests indicated that, to be killed in a reasonable time (15 to 20 days), ammocetes should be exposed to a concentration of at least 80 p.p.b. (table 3). Because lack of time prohibited more extensive study, a concentration of 100 p.p.b. was chosen for the treatment.

TABLE 3.--Results of bioassays with toxaphene on lamprey ammocetes, October 1961

[Temperature, 58°-66° F.]

Size of test container	Concentration of toxaphene	Exposure time	Mortality
	P.p.b.	Days	Percent
200 gallons.....	50	17	0
20 gallons.....	40	19	10
20 gallons.....	80	16	100
20 gallons.....	120	9	40
20 gallons.....	120	12	100

APPLICATION OF CHEMICALS

The Sucker River was re-treated with the selective larvicide 3-trifluoromethyl-4-nitrophenol (TFM), October 19-22, 1961, to prevent immediate recontamination of East Bay by young lampreys from upstream.

Treatment of East Bay with toxaphene began on October 24. Eighty-five gallons of this toxicant, the amount needed to give a concentration of 100 p.p.b. through the entire volume, were distributed systematically over the surface of the lake. Simultaneously, toxaphene was added (by means of a portable fuel-pump feeder--Anderson, 1962) into the river above East Bay at a rate to produce 100 p.p.b. in the water entering East Bay. This pump operated continuously for 14 days.

Eleven days after the distribution of toxaphene began, 5 more gallons were applied to the surface of a small backwater at the southeast end of the lake and to the shoreline on the east end of the lake. A few ammocetes had been observed swimming in those areas the previous day.

Before treatment, 10 lamprey larvae were placed in each of 15 cages (11 cages in East Bay, 2 in the connecting channel to West Bay, and 2 in West Bay near the mouth of the channel--fig. 1). The condition of these larvae and the number dead were recorded at intervals from October 22 until November 26, when cold weather forced termination of observation.

Equipment was not available for quantitative determination of toxaphene during this experiment. Assuredly, it would have been advantageous to know the toxaphene concentration at

different intervals after application. The work of others would seem to indicate that concentrations reached may have been considerably less than those desired (Kallman, Cope, and Navarre, 1962).

IMMEDIATE EFFECTS OF THE TOXICANT

Small fish were observed surfacing and dying the day after treatment began. Mortality of fish increased daily, reached a peak during the third and fourth days, and ended shortly after. The area was kept under observation during the treatments, and estimates were recorded of the number and species of dead fish. These estimates were biased by recovery of fish by fishermen. Approximately 50 to 80 large rainbow trout (more than 12 inches long) and 25 northern pike were the only game fish of appreciable size observed. Yellow perch were killed in large numbers (3,000 to 4,000), but almost none were over 6 inches long. Several hundred white suckers, several thousand minnows, and a few small rainbow trout, round whitefish, walleye, burbot, and rock bass were seen.

The first dead larval lampreys were seen on the fourth day after treatment began. Their numbers were small in relation to the estimated size of the population.

Ammocetes were slow to emerge from the mud in the bottom of the test cages. Only a few individuals in each container in East Bay had appeared by the end of 4 days. All specimens had emerged by the sixth day and approximately one-third were dead. Most of the remainder were near death.

After emergence from the bottom, some larval lampreys swam aimlessly for a short time, but most lay contorted on the bottom and gave only an occasional twitch. They remained in this condition to the end of the daily observations. Many lampreys gave indications of hemorrhage around the gills, sides, and anus. All ammocetes showed loss of pigmentation.

Daily observations of the caged lampreys were continued for 14 days. During this period, 6 cages were lost in storms. Of the original 90 specimens in the remaining 9 cages, all in 4

cages were dead and 33 in 5 cages were alive at the end of 14 days. Observations were terminated at the end of 36 days of exposure. Two ammocetes were then still alive.

POSTTREATMENT SURVEYS

A riffle fyke net, fished in the connecting channel between East and West Bays after the treatment until November 30, captured no lamprey ammocetes. It was reset in the same location March 15 and fished without interruption until November 26, 1962. During this period, only 1 American brook lamprey ammocete was taken.

The electric beam trawl was dragged in East Bay in May and August 1962, for a total towing time of 113 minutes. No lampreys were taken. Trawling in West Bay near the mouth of the channel in 1962 yielded 7 ammocetes (5 sea lampreys and 2 American brook lampreys) in 79 minutes.

It is evident from these data that toxaphene brought about nearly complete eradication of ammocetes in East Bay. The two test animals that were still alive 36 days after treatment indicated that mortality may not have been complete, but it is probable that larvae not dead at the end of the treatment period had received a lethal dose and subsequently died.

The fish population was sampled again 1 year after the experiment to determine the extent of recovery from the toxaphene. Nets fished 1 night at each of three 1961 locations captured 68 fish, including 4 rainbow trout over 12 inches, 2 northern pike, and 20 white suckers (table 2). The population of yellow perch was sharply lower than a year earlier, but the number of immature rainbow trout had increased.

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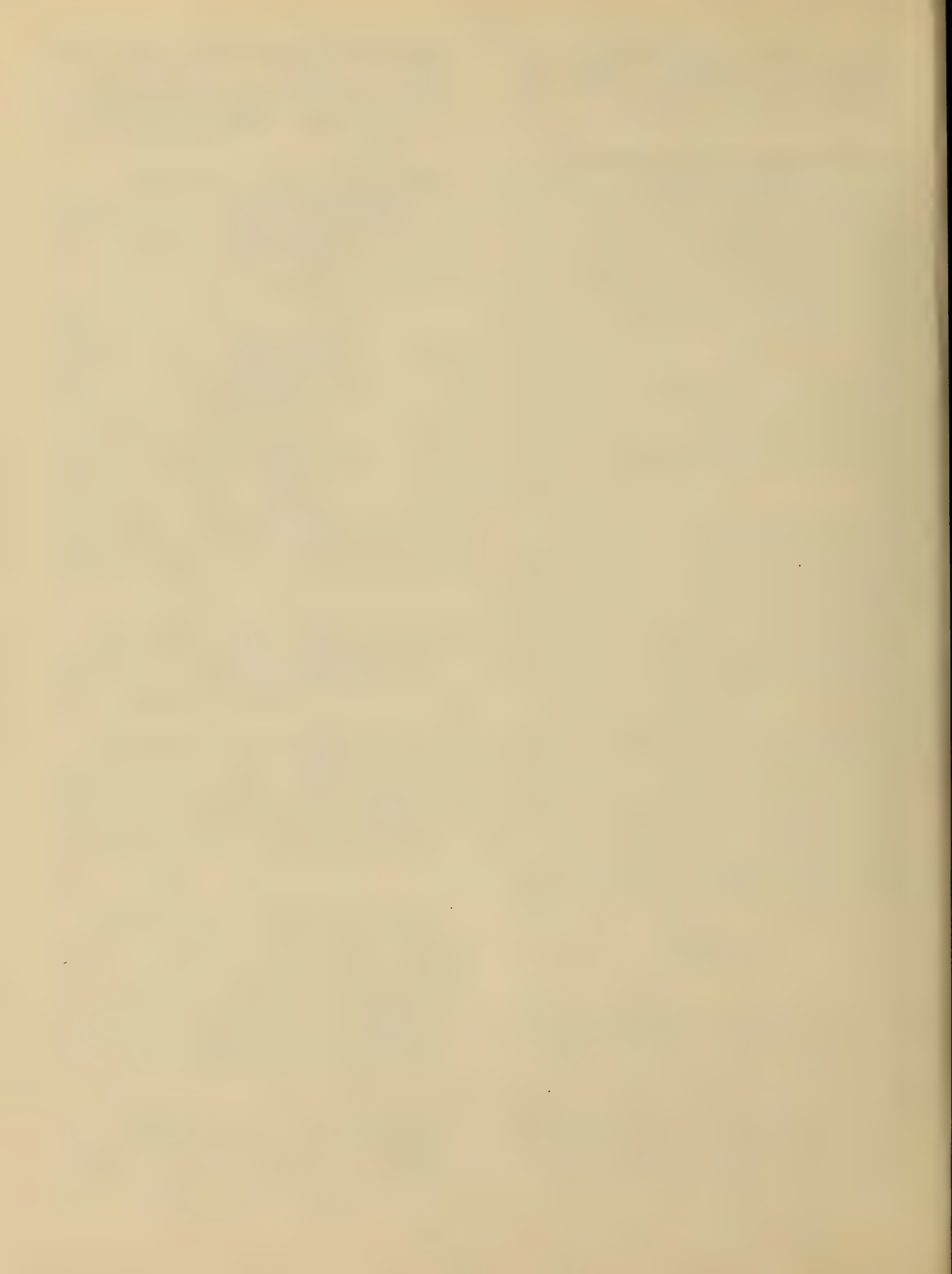
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INVESTIGATIONS IN FISH CONTROL

**8. Effects of Toxaphene on Fishes and Bottom Fauna
of Big Kitoi Creek, Afognak Island, Alaska**

By William R. Meehan and William L. Sheridan
Alaska Department of Fish and Game



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EFFECTS OF TOXAPHENE ON FISHES AND BOTTOM FAUNA OF BIG KITOI CREEK, AFOGNAK ISLAND, ALASKA

By William R. Meehan and William L. Sheridan
Alaska Department of Fish and Game

Abstract.--Big Kitoi Creek, on Afognak Island, Alaska, was treated with toxaphene in July 1961 to remove sculpins predaceous on pink salmon fry. Dispersion and penetration of toxaphene into the streambed were determined, as well as time required for detoxification. The population of sculpins in the creek before treatment was estimated at 30,000, of which 82 percent were in the size range considered predaceous on pink salmon fry. Extent of predation was determined by examination of stomachs of 180 sculpins. Considering the rate of predation, it was estimated that, of $847,500 \pm 418,600$ fry in the gravel 3 months before treatment, 12 percent may have been eaten by sculpins before the fry migrated to salt water. Bottom fauna decreased in numbers and weight after the toxaphene treatment: insects were completely eradicated; some other invertebrate groups were not completely eliminated. Posttreatment recruitment of bottom fauna began later in the summer; a year later the pretreatment levels of biomass had not yet been reached. Species composition of bottom fauna a year after treatment differed somewhat from that before treatment. Assuming that, if the creek had not been treated, 30,000 sculpins would have been present in the spring of 1962, then the treatment possibly saved approximately 135,000 pink salmon fry in 1962.

Pink salmon (Oncorhynchus gorbuscha) and chum salmon (O. keta) deposit their eggs in the gravel of coastal streams in Alaska from July to October. Eggs incubate through the winter, and fry migrate into salt water the following spring. During downstream migration, fry of both species are from 26 to 36 millimeters long and are subject to predation both in streams and in estuaries.

Since pink and chum salmon go to sea almost immediately after they emerge from streambed gravels, they usually are not dependent on a food supply while in fresh water. Coho salmon (O. kisutch), on the other hand, remain in fresh water for 1 or 2 years before going to sea;

hence, the food supply may sometimes limit their survival.

The coastrange sculpin (Cottus aleuticus) is one of the chief predators on salmon fry in fresh-water streams. Because there were large numbers of C. aleuticus as well as an annual run of several thousand pink salmon in Big Kitoi Creek, a study was carried out in 1961 and 1962 to determine (1) the extent of predation by sculpins on pink salmon fry, (2) the effects of toxaphene on the sculpins, and (3) the effects of toxaphene on the bottom fauna.

If predation by sculpins was significant, eradication of the sculpin population would relieve downstream migrant pink salmon fry of this cause of mortality. If the bottom fauna was at the same time eradicated, and the rate of

Present address of the authors: U.S. Forest Service, Box 1631, Juneau, Alaska.

recovery was slow, production of resident fish such as coho salmon and trout would be limited.

Predation by sculpins probably depends on the species of sculpin and availability of salmon fry. Bailey (1952) reported that food studies on *Cottus bairdi* by Ricker (1934), Koster (1937), and Dineen (1951) indicated that this species was not a serious game fish predator. Bailey's study of *Cottus bairdi* in Montana agreed with these findings. On the other hand, Robertson (1949) reported that sculpins (species not mentioned) exacted the greatest toll of sockeye salmon fry migrating from tributary streams into Port John Lake in British Columbia. One sculpin, 4-1/2 inches long, contained 38 fry, and the average was 13 fry per sculpin. Pritchard (1936) determined that the average consumption of salmon fry by sculpins in McClinton Creek in British Columbia for 1931 and 1933 respectively was 0.8 and 1.5 fry per sculpin. Hunter (1959) determined that in Hooknose Creek, Port John, British Columbia, *C. aleuticus* and *C. asper* fed almost exclusively on pink and chum salmon fry during downstream migration. Hunter calculated that numbers of pink and chum fry consumed by sculpins alone (1948 to 1957) ranged from 73,868 in 1950 to 276,833 in 1948. Hikata and Nagasawa (1960) reported that of 442 *C. nozawae* stomachs examined in Memu Stream, Tokachi River system, 5.4 percent contained salmon fry. Patten (1962) reported on predation upon coho salmon fry by *C. perplexus* and *C. rhotheus*. Hence, where fresh-water sculpins have access to salmon fry, they can be serious predators. In years when few pink salmon fry are produced in a stream a greater proportion would be eaten, perhaps enough to keep a low spawning run depressed (Neave, 1953).

STUDY AREA

Big Kitoi Creek originates at the outlet of Big Kitoi Lake, a 356-acre lake on Afognak Island, Alaska (fig. 1). The creek flows approximately 2,000 feet before it enters salt water; only the lower half of the stream is accessible to anadromous fishes, because two falls prevent passage. Average gradient of the stream below the falls is 2.3 percent, and the streambed consists of rocks up to 18 inches in diam-

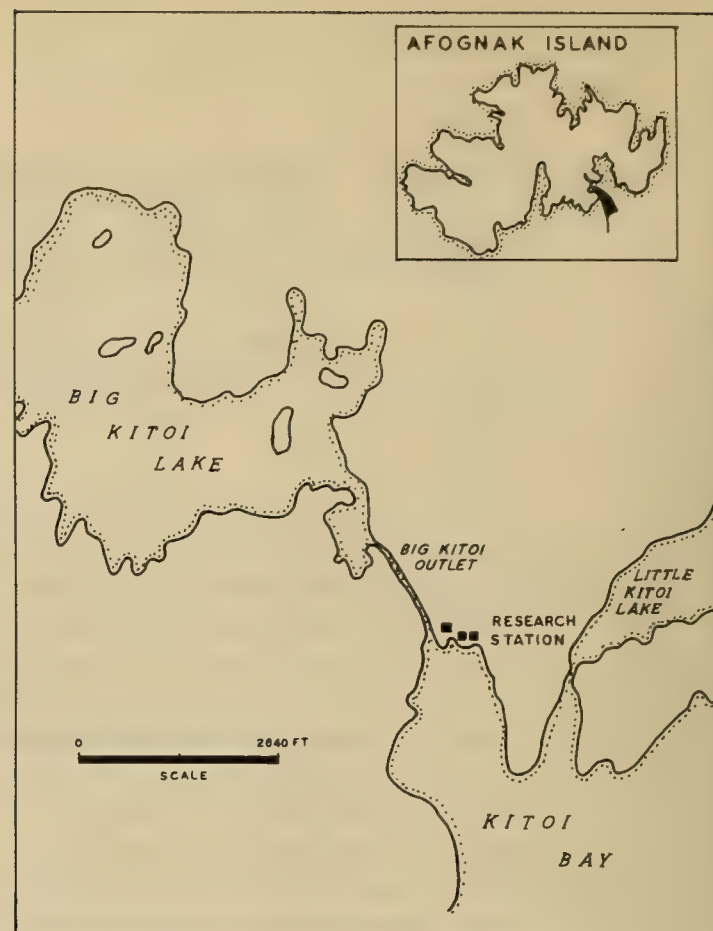


Figure 1.--Location of study area, Afognak Island, Alaska.

eter. At least 80 percent of the particles comprising the streambed are larger than 3 millimeters. The stream averages 20 feet in width and is from 1 to 3 feet deep during normal water conditions.

The stream was marked off in 100-foot sections for sampling, with section I at the mouth and section 10 ending immediately below the first falls. Only 700 feet proved to be usable for the bottom fauna study (sections 3 through 9), since the lower two sections were saline and section 10 was too swift. Section 3 was flooded at each high tide; tidal influences were also observed in section 4, and at extremely high tides in section 5. Above section 5, the stream narrowed and was swifter than in the lower sections.

In addition to sculpins and pink salmon, other fishes in Big Kitoi Creek in varying numbers and at intervals were Dolly

Varden (*Salvelinus malma*), threespine stickleback (*Gasterosteus aculeatus*), coho salmon, rainbow trout (*Salmo gairdneri*), and to a much lesser extent sockeye salmon (*O. nerka*), crescent gunnel (*Pholis laeta*), and smelt (*Osmeridae*).

METHODS

FISH

The population of sculpins in Big Kitoi Creek before poisoning was estimated in two ways: (1) By flushing random 4-square-foot areas with an air-water jet directed into the streambed (McNeil, 1960) and dislodging sculpins into a collecting net, and (2) by tag and recovery. In the latter case 255 sculpins were collected with a 110-volt a.c. electroshocker, tagged, and released back into the creek.

To evaluate the seriousness of predation in Big Kitoi Creek, rate of digesting was first studied by placing 37 sculpins in a glass aquarium, starving them for 24 hours (Darnell and Meierotto, 1962), then furnishing them with an abundance of pink salmon fry for a short time. Stomachs from sculpins taken in the creek were examined, and condition of pink salmon fry compared with the laboratory specimens.

Extent of predation was studied by collecting 180 sculpins with an electroshocker on May 10, 11, and 12, 1961, and examining stomachs. During this period, downstream pink salmon fry migration was at its peak.

BOTTOM FAUNA

Bottom organisms were sampled periodically before and after toxaphene treatment by means of a Surber square-foot bottom sampler. Three samples were taken in each section once a month. The sampling sites were selected by use of a table of random numbers at the beginning of the study, and subsequent samples were taken adjacent to the initial sampling sites so that the same type of substrate was sampled each month in each section. These samples were preserved in formal-alcohol for future analysis. Preservative was composed of equal parts by volume of 70-percent ethyl alcohol and

5-percent formalin. Each sample was then sorted, the organisms were identified, and the number in each group was recorded. Later, the total fauna of each square-foot sample was blotted for 1 minute and immediately weighed on an analytical balance. Shells of live mollusks were included in the weights; empty shells and cases of Trichoptera larvae were not included. Weights were recorded to the nearest milligram. These data were later transferred to punchcards for tabulation.

APPLICATION OF TOXAPHENE

The stream was treated with toxaphene (Agricultural Cooper-Tox No. 6) on July 5 and 6, 1961. At this time all pink salmon fry had migrated to sea, and returning pink salmon adults had not yet entered the creek.

Two 5-gallon cans of toxaphene were placed at the top of a 12-foot falls, immediately above Section 10, and were siphoned directly into the creek through a rubber tube and metered with an adjustable clamp. The rate of flow was determined by means of a graduated cylinder and stopwatch. Concentrations were calculated on the basis of amount of toxaphene mixed with known discharge (13.1 c.f.s.). Toxaphene was applied for 18.5 hours, with an average concentration of 1.5 p.p.m.

Immediately after treatment, all visible dead fish were counted in sections 3, 4, and 5. Some rocks were turned over and the streambed examined, because many sculpins remained under rocks after they had died.

Heavy concentrations of toxaphene caused water in the stream to turn a milky color. Although it appeared that thorough mixing was achieved, fluorescein dye was inserted at the treatment point to make mixing easier to observe. Observations of dye showed complete mixing from the falls to tidewater, even in small pools and backwater eddies.

To determine the extent of penetration of toxicant into streambed gravels, plastic standpipes were placed 7 or 8 inches deep at six cross-sectional stations. Vaux (1962) and Sheridan (1962) describe the interchange between surface stream water and water in the

gravel of the streambed. Based on these findings alone, we can assume penetration of toxicant into the gravel. In corroboration of the assumption, after dye was released upstream its presence was detected in samples of water withdrawn from the standpipes. Therefore, as assumed, diluted toxicant penetrated to a depth of at least 7 inches into the streambed.

Toxicity of stream water was evaluated by placing live fish (sculpins and salmonids from a nearby stream) in small wire-screen minnow traps up and down the stream and observing effects at periodic intervals during and after treatment.

RESULTS

FISH

Of the 255 sculpins tagged, 13 were recovered in a sample of 2,000 sculpins collected after poisoning. The population estimate by this method is then approximately 40,000 sculpins in the creek below the falls before poisoning. At approximately the same time 95-percent confidence limits for the population determined by hydraulic displacement were $37,957 \pm 9,265$. We feel that, based on the above population estimates, 30,000 sculpins is a reasonable estimate of the total number of sculpins in the stream. Of these 30,000, 82 percent fall in the size range 5.0 cm. to 11.9 cm. (based on a sample of 597 sculpins measured after treatment).

Sculpins maintained in the aquarium for determination of rate of digestion of pink salmon fry were reluctant to feed in captivity, but enough did eat so that when sacrificed at periodic intervals after feeding, condition of consumed fry could be determined. Seven hours after consumption, pink salmon fry were readily detectable as such. After 20 hours, fry were mostly a digested mass, almost unrecognizable as fish. This means that pink salmon fry (recognizable as such) found in sculpin stomachs in the morning were most likely eaten the preceding night.

Of 180 sculpins collected before poisoning, 25 contained an average of 2.1 fry, or an overall

average of 0.14 fry per sculpin. The smallest sculpin (total length) which contained fry was 2.7 cm. and the largest was 8.7 cm. We think that this example of consumption of a fry by a predator no larger than the prey was an isolated instance. Therefore, in the following calculations of extent of predation, this one instance is disregarded.

Using a method similar to that of Hunter (1959), we multiplied 30 days (duration of most intense pink salmon fry migration) \times number of sculpins capable of consuming fry (24,600) \times rate of predation (0.14) and found that 103,320 pink salmon fry were possibly consumed by sculpins during the 1961 spring migration. Previously we estimated $847,500 \pm 418,600$ fry in the gravel in March; hence, 12 percent of the mean estimated number of pink salmon fry may have been eaten by sculpins.

In addition to sculpins, other fish were observed dead as follows: Dolly Vardens, 43; rainbow trout, 3; coho fingerlings, 74; and gunnells, 6.

BOTTOM FAUNA

Bottom organisms decreased in number and weight after toxaphene treatment in July 1961 (tables 1 and 2). Before treatment, the species composition of Big Kitoi Creek bottom fauna changed rapidly as the influence of salt water decreased, or as areas further upstream were considered. The various species of midges (Tendipedidae), for example, made up a much larger part of the bottom fauna upstream than in sections 3 and 4 where tidewater influences were pronounced. Conversely, the brackish-water-inhabiting forms such as the amphipods and isopods decreased in relative abundance

TABLE 1.--Number and weight of organisms per square foot before and after poisoning

Section	Before poisoning (Spring 1961)		After poisoning			
			Summer 1961		Spring 1962	
	Number	Weight (grams)	Number	Weight (grams)	Number	Weight (grams)
3.....	336.0	1.383	94.6	0.414	156.9	0.206
4.....	115.5	0.310	50.0	0.069	62.3	0.082
5.....	134.6	0.142	77.8	0.287	33.7	0.057
6.....	71.5	0.167	45.6	0.045	108.3	0.187
7.....	--	--	33.0	0.121	134.2	0.253
8.....	--	--	--	--	115.6	0.186
9.....	--	--	7.8	0.016	66.2	0.345
Mean.....	164.4	0.501	50.7	0.136	96.8	0.188

TABLE 2.--Analysis of variance for difference in number and weight of organisms per square foot before and after treatment

[July to September]

	Degrees of freedom	Sum of squares	Mean square	F
Mean number of organisms per square foot:				
Between treatment.....	1	144,327	144,327	15.6**
Within treatment.....	60	554,134	9,236	
Total.....	61	698,461		
Mean weight of organisms per square foot:				
Between treatment.....	1	8,783	8,783	48.0**
Within treatment.....	58	10,614	0.183	
Total.....	59	19,397		

**Significant at 1 percent level.

with a similar transition to a completely freshwater environment. Midges were the most important group throughout the stream; the crustaceans (Amphipoda and Isopoda) were also relatively abundant, although primarily in the intertidal sections.

Treatment of the stream with toxaphene had several effects on the bottom fauna. The insect groups succumbed completely and more rapidly than other invertebrates, such as clams and snails, which were the last groups to be affected and which were never completely eliminated. Although the July sampling showed a complete lack of live insects, the insects began to reinvade the stream later in the summer. Emergence of adult insects contributed to this elimination of insects in the July sampling, as evidenced by a few shed larval cases and pupal skins; however, most of the insects were killed before emergence. Amphipods and isopods in the intertidal sections were eventually completely eradicated, although these two groups were still present in the July sampling, indicating that they were more resistant. The upstream sections became barren of bottom fauna before the lower and intertidal sections.

DETOXIFICATION AND RECOVERY OF CREEK

Fish.--Bioassay with coho and sockeye smolts, threespine stickleback, and sculpins showed that the stream became nontoxic after 21 days. In addition, Dolly Vardens moved into the stream from salt water early in August and remained throughout the salmon spawning run.

Final evidence that Big Kitoi Creek became free from toxicant in a short time was the success of pink salmon spawning in the stream after treatment. Pinks deposited eggs from August 25 until early October, 1961; hence, spawning first occurred 50 days after treatment. Sampling of eggs in the streambed in November 1961 revealed a very high survival, and subsequent sampling of preemergent fry in March 1962 gave a mean estimate of 1,124,000 fry, or 44 percent egg-to-fry survival.

Bottom fauna.--When initial recruitment of organisms began later in the summer, the upstream sections were the first to show an influx. This is probably due to the drifting in of organisms from the lake and stream above the falls area (Waters, 1961). The partially eradicated mollusk populations were the first to show a marked increase after treatment, and the insect groups were slowest to appear in quantity. One year later the pretreatment levels of biomass, in terms of weight and number of organisms per square foot of streambed, had not been reached. The species composition of the bottom fauna almost 1 year after treatment was somewhat different from that before treatment. The noninsect forms, including annelids, were the dominant forms throughout the study area, although the tendipedids were still the most numerous insect form. In the intertidal sections, annelid worms were abundant while the previously numerous amphipods and isopods were absent. The annelids were becoming reestablished in the most saline environment and were gradually expanding to the less saline areas. The mollusks (Gastropods, Pelecypoda), which were never completely eradicated, became established in greater magnitude after treatment but occupied the same general area as before treatment.

DISCUSSION

Results of treating Big Kitoi Creek with toxaphene show that this was an economical (cost of toxaphene for Big Kitoi Creek was \$28) and effective way to control predation on salmon fry in this stream.

Although the method described in this report lends itself to eradication of undesirable fish in streams, it cannot be used where there are

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resident populations of trout and/or salmon which should be maintained. Both the desirable fish and their food supply would be eradicated. On the other hand, there are streams in Alaska which support only pink and chum salmon and these streams are ideal for treatment. For example Noerenberg (1960) reported that in Prince William Sound pink and chum salmon comprise 90 percent of the run, sockeye salmon 10 percent. Coho salmon are of little importance and no known spawning runs of chinook salmon (*O. tshawytscha*) or steelhead trout (*Salmo gairdneri*) exist.

Assuming that 30,000 sculpins would have been present in Big Kitoi Creek in the spring of 1962 if treatment had not occurred, it is possible that 12 percent, or approximately 135,000 pink salmon fry, were saved in 1962 by the treatment.

SUMMARY

Part of Big Kitoi Creek, on Afognak Island, Alaska, was treated with toxaphene to determine (1) the extent of predation by sculpins on pink salmon fry in the stream, (2) the effects of toxaphene on the sculpins, and (3) the effects of toxaphene on the bottom fauna.

The estimated population of sculpins before treatment was 30,000. Approximately 100,000 pink salmon fry could have been taken by sculpins in 1961. Treatment with toxaphene eliminated sculpins and other fishes in a 1,000-foot section of the stream. Sculpins were not observed up to 1 year later.

Insects were eliminated, and populations of other invertebrate forms were greatly reduced. Recruitment of bottom organisms began shortly after treatment, but 1 year after treatment the pretreatment levels of bottom fauna biomass had not been reached. In general, the various groups of organisms were reestablished in the same areas they inhabited before treatment; in a few cases major groups have been replaced by different forms which were less well represented before treatment.

In this case we feel that the treatment of Big Kitoi Creek with toxaphene was an economical and effective means of reducing predation on pink salmon fry.

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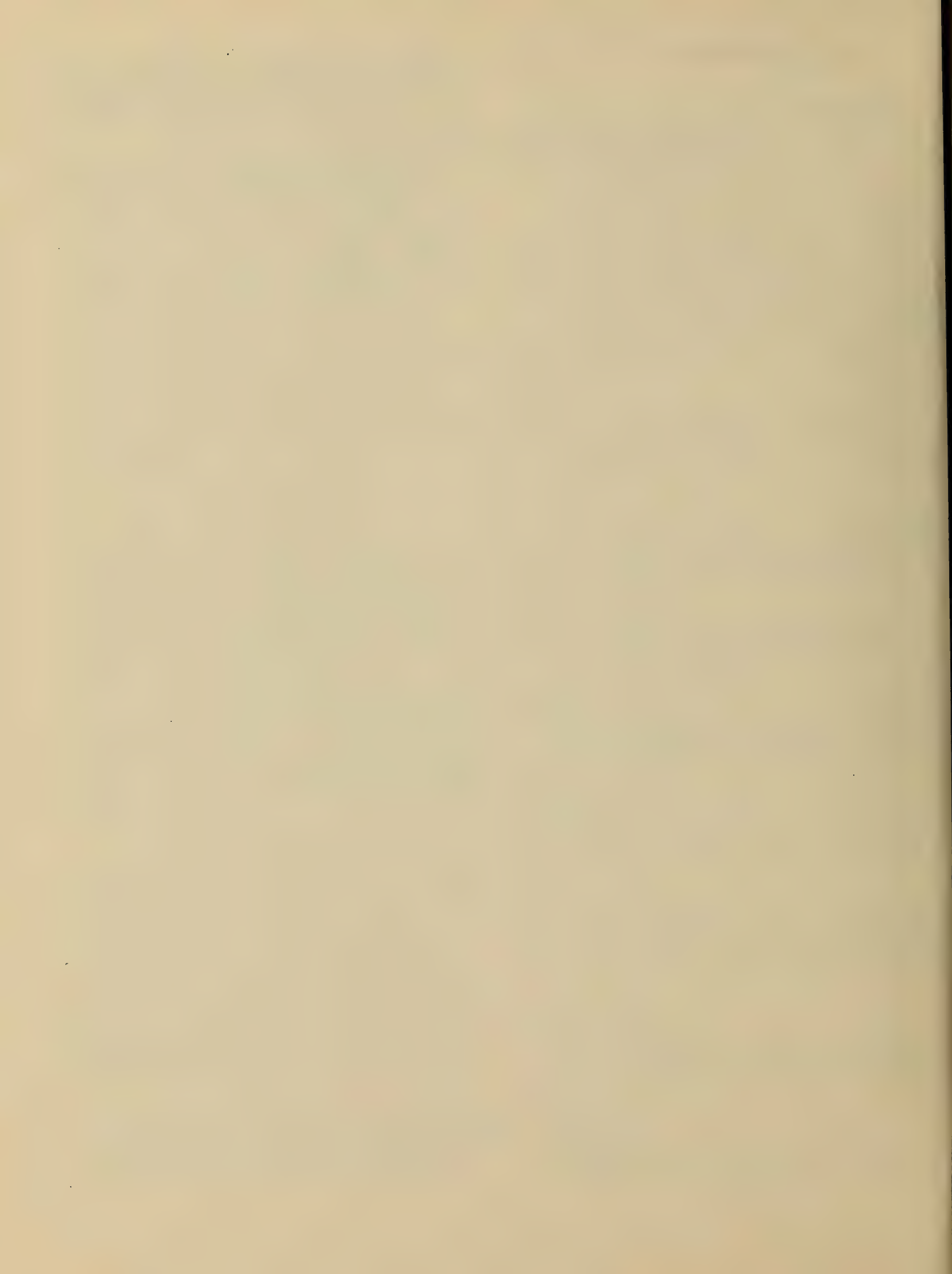
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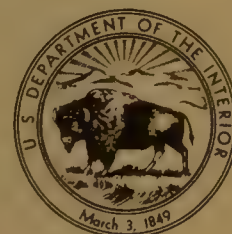
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INVESTIGATIONS IN FISH CONTROL

9. Relation of Chemical Structure to Fish Toxicity
in Nitrosalicylanilides and Related Compounds
10. Evaluation of p, p'-DDT
as a Reference Toxicant in Bioassays
11. Evaluation of an Electronic Method of
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United States Department of the Interior
Fish and Wildlife Service
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INVESTIGATIONS IN FISH CONTROL

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11. Evaluation of an Electronic Method of Measuring Hematocrits of Fish, by Richard A. Schoettger and Arnold M. Julin. 1966. 11 p. (Resource Publication 15.)

Fish Control Laboratories
Bureau of Sport Fisheries and Wildlife
U.S. Department of the Interior
P. O. Box 862
La Crosse, Wisconsin 54602

INVESTIGATIONS IN FISH CONTROL

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(Resource Publication 15, p. 1-11)

Washington, D.C.
February 1966



United States Department of the Interior, Stewart L. Udall, *Secretary*
Stanley A. Cain, *Assistant Secretary for Fish and Wildlife and Parks*
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Bureau of Sport Fisheries and Wildlife, John S. Gottschalk, *Director*

9. Relation of Chemical Structure to Fish Toxicity in Nitrosalicylanilides and Related Compounds

By Charles R. Walker, Chemist
Fish Control Laboratory
Bureau of Sport Fisheries and Wildlife
La Crosse, Wisconsin

Roland J. Starkey, Microbiologist
Ben Venue Laboratories, Inc.
Bedford, Ohio

Leif L. Marking, Chemist
Fish Control Laboratory
Bureau of Sport Fisheries and Wildlife
La Crosse, Wisconsin



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RELATION OF CHEMICAL STRUCTURE TO FISH TOXICITY IN NITROSALICYLANILIDES AND RELATED COMPOUNDS

By Charles R. Walker, Chemist
Fish Control Laboratory, La Crosse, Wis.

Roland J. Starkey, Microbiologist
Ben Venue Laboratories, Bedford, Ohio

and Leif L. Marking, Chemist
Fish Control Laboratory, La Crosse, Wis.

ABSTRACT.--Relations between chemical structures of salicylanilides and benzanilides and their toxicity to rainbow trout and goldfish were evaluated in standard, static bioassays. Single and multiple substitutions of alkyl-, nitro-, and halo- groups were tested. Substitution of hydroxy- at ortho (2) accompanied by nitro- at meta (3) or sub-meta (5) on the benzoic acid moiety are basic requirements for toxic activity against fish. Halogenation at para (4') markedly affects the selective toxicity of the compounds to either rainbow trout or goldfish.

A search for potent piscicides is a primary function of the Fish Control Laboratory at La Crosse, Wis., and the Southeastern Fish Control Laboratory at Warm Springs, Ga. Candidate compounds are bioassayed to determine their potentials as selective or general fish toxicants. Among the more toxic compounds studied to date is antimycin A (Walker, Lennon, and Berger, 1964). Strong (1958) described the capacity of antimycin A to inhibit the electron-transport system in higher animals, and Derse and Strong (1963) suggested that the antibiotic may be useful as a toxicant highly selective to fish. Since antimycin A is a 3-formamidosalicylic lactone, interest was stimulated in the chemical and biological activities of N-substituted nitrosalicylates and formidosalicylates. Dickie et al. (1963) demonstrated that certain nitro- and formido-substitutions were significantly more active biologically than others. We elected to investigate certain salicylic acid derivatives to detect relations between chemical structure and biological activity against fish.

Investigations with other test organisms have shown some relation between chemical structure of salicylanilides and toxicity. Taborsky et al. (1959, 1962, 1963) described the importance of specific substitutions to antimicrobial activity. Baichwal et al. (1960a) described fungicidal activities, and Schraufstatter (1962) demonstrated relations between structure and molluscicidal properties. Recently, Starkey and Howell (1965) pointed out the importance of molecular structure of salicylanilides to selective toxicity to the sea lamprey (Petromyzon marinus).

The object of this study was to ascertain the structural requirements of compounds to produce toxicity in two fishes. The compounds included certain substituted nitrosalicylanilides and benzanilides with consideration given to single and multiple substituents, and in particular to the locus of the nitro and halogen groups. The fish were rainbow trout (Salmo gairdneri) and goldfish (Carassius auratus).

METHODS AND MATERIALS

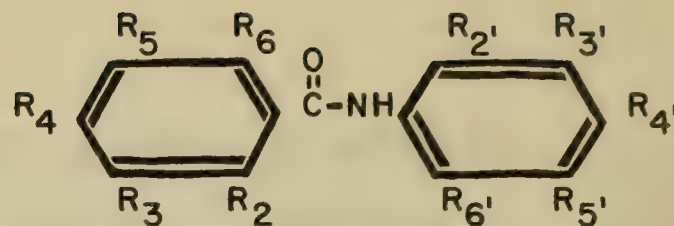
The bioassay methods used were described by Lennon and Walker (1964) for the evaluation of fish control agents. All bioassays were conducted in reconstituted water at 12°C. The rainbow trout were acquired from the Manchester, Iowa, National Fish Hatchery and acclimated to the laboratory water supply. The mean LC_{50} of *p,p'*-DDT for them was 6.8 parts per billion, with a range from 1.9 to 14.0 p.p.b. (table 1). The goldfish were obtained from the Lake Mills, Wis., National Fish Hatchery and weighed from 0.7 to 2.5 grams. The mean LC_{50} of *p,p'*-DDT for them was 41.2 p.p.b., with a range from 27.0 to 76.0 p.p.b. (table 1).

TABLE 1.--Comparative sensitivities of different lots of fish to the reference standard *p,p'*-DDT expressed as the LC_{50} value in p.p.b.

Species and lot	Average weight (grams)	Date bioassayed	LC_{50} (p.p.b.)
Rainbow trout:			
Lot 16a.....	0.92	Mar. 24, 1964	14.0
Lot 17.....	0.92	Apr. 10, 1964	4.6
Lot 18C.....	0.20	May 21, 1965	1.9
Mean LC_{50}			6.8
Goldfish:			
Lot 1.....	2.50	Mar. 4, 1964	76.0
Lot 19.....	2.38	Mar. 31, 1964	27.0
Lot 185.....	0.70	Apr. 3, 1964	32.0
		June 27, 1965	29.7
Mean LC_{50}			41.2

Toxicity of the compounds was determined at three concentrations (0.1, 1.0, and 10 parts per million) on rainbow trout and goldfish in 2.5- or 15-liter glass containers. Ten fish were bioassayed at each concentration, and the effects of the chemicals upon the fish were recorded at 0.25, 0.5, 1, 3, 24, 48, 72, and 96 hours. The data presented in this paper are confined to observations made at the end of 3 and 48 hours, which appeared to be the critical times for differentiating the gross acute toxic effects.

Ben Venue Laboratories, Inc., Bedford, Ohio, furnished substituted salicylanilides and benzanilides for evaluation of relations between structure and piscicidal activity (fig. 1 and table 2). Substituted nitrosalicylanilides of the general formula depicted in figure 1 have been documented (Taborsky et al., 1959; Taborsky and Starkey, 1963; and an unpublished manuscript "Some substituted salicylanilides"



SALICYLANILIDES

R_2 = OH accompanied by one or more of the following:

R_3 = NO_2 , and/or R_5 = NO_2 , Cl, Br, or acetylamine

$R_2' - R_6'$ = NO_2 , F, Cl, Br, I, CH_3 , OCH_3 , C_2H_5 , and/or phenylazo in one or more positions.

BENZANILIDES

$R_2 - R_5$ = NO_2 , Cl, and/or OH with the following:

$R_2' - R_6'$ = NO_2 , Cl, Br, I, CH_3 , OCH_3 , and C_2H_5 in one or more positions.

Figure 1.--General structure of substituted salicylanilides and benzanilides.

by Roland J. Starkey). They can be arbitrarily divided into five groups based upon substituents in the aniline moiety: (1) halonitrosalicylanilides; (2) halonitrosalicylotoluidides; (3) nitrosalicyloxyliidides; (4) nitrosalicylanisidides; and (5) a miscellaneous group including 4'-phenylazo-3-nitrosalicylanilide and 2'-ethyl-3-nitrosalicylanilide.

Salicylanilides are synthesized by the condensation of salicylic acid or a derivative with aniline or a derivative thereof. This produces a biphenyl molecule with a CONH bridge and is characterized by the presence of an ortho phenolic hydroxyl group (OH) in the acid moiety. Benzanilides, also biphenyl in structure with the CONH bridge but devoid of the hydroxyl group, are prepared in the same manner with a derivative of benzoic acid.

3-nitrosalicylanilides, in addition to a hydroxyl group, possess a nitro (NO_2) group at the 3 (meta) position of the acid moiety

TABLE 2.--Compounds used in evaluating the relation of chemical structure to fish toxicity

Compound	Chemical name ¹	Compound	Chemical name ¹
1.	3-nitrosalicylanilide	36.	2'-nitro-p-salicylanisidide
2.	2'-iodo-3-nitrosalicylanilide	37.	2',3-dinitro-m-salicylanisidide
3.	2'-ethyl-3-nitrosalicylanilide	38.	2'-chloro-3,4'-dinitrosalicylanilide
4.	3'-chloro-3-nitrosalicylanilide	39.	3-nitrosalicylanilide
5.	3'-bromo-3-nitrosalicylanilide	40.	benzanilide
6.	3'-iodo-3-nitrosalicylanilide	41.	3'-chloro-3-hydroxybenzanilide
7.	4'-chloro-3-nitrosalicylanilide	42.	4'-chloro-3-hydroxybenzanilide
8.	4'-bromo-3-nitrosalicylanilide	43.	2'-chloro-2-nitrobenzanilide
9.	4'-iodo-3-nitrosalicylanilide	44.	3'-chloro-2-nitrobenzanilide
10.	4'-phenylazo-3-nitrosalicylanilide	45.	4'-chloro-2-nitrobenzanilide
11.	2'-chloro-5-nitrosalicylanilide	46.	4'-bromo-2-nitrobenzanilide
12.	3'-fluoro-5-nitrosalicylanilide	47.	3'-chloro-2-nitro-p-benzotoluidide
13.	4'-chloro-5-nitrosalicylanilide	48.	3-nitrobenzanilide
14.	4'-iodo-5-nitrosalicylanilide	49.	2'-chloro-3-nitrobenzanilide
15.	3'-chloro-5-acetamidosalicylanilide	50.	3'-chloro-3-nitrobenzanilide
16.	2',4',6'-trichloro-3-nitrosalicylanilide	51.	3'-chloro-3-nitro-p-benzotoluidide
17.	3',4'-dichloro-3-nitrosalicylanilide	52.	2'-chloro-4-nitrobenzanilide
18.	2',5'-dibromo-3-nitrosalicylanilide	53.	3'-chloro-4-nitrobenzanilide
19.	4'-chloro-5-bromo-3-nitrosalicylanilide	54.	5'-chloro-4-nitrobenzanilide
20.	4',5-dibromo-3-nitrosalicylanilide	55.	p-chlorobenzanilide
21.	3-nitro-2',3'-salicyloxyldide	56.	3,5-dinitrobenzanilide
22.	3-nitro-2',4'-salicyloxyldide	57.	2'-chloro-3,5-dinitrobenzanilide
23.	3-nitro-2',5'-salicyloxyldide	58.	3'-chloro-3,5-dinitrobenzanilide
24.	3-nitro-2',6'-salicyloxyldide	59.	3'-bromo-3,5-dinitrobenzanilide
25.	3'-chloro-3-nitro-g-salicylotoluidide	60.	4'-bromo-3,5-dinitrobenzanilide
26.	4'-chloro-3-nitro-g-salicylotoluidide	61.	4'-iodo-3,5-dinitrobenzanilide
27.	2'-chloro-3-nitro-p-salicylotoluidide	62.	2'-fluoro-3,5-dinitrobenzanilide
28.	6'-chloro-3-nitro-g-salicylotoluidide	63.	3,5-dinitro-g-benzotoluidide
29.	3,5'-dinitro-g-salicylotoluidide	64.	3,5-dinitro-2',3'-benzoxylidide
30.	2',3-dinitro-p-salicylotoluidide	65.	3'-chloro-3,5-dinitro-p-benzotoluidide
31.	4'-bromo-3-nitro-g-salicylotoluidide	66.	5'-chloro-3,5-dinitro-g-benzotoluidide
32.	5'-chloro-3-nitro-g-salicylanisidide	67.	3'-chloro-3,5-dinitro-g-benzotoluidide
33.	4'-chloro-2',5'-dimethoxy-3-nitrosalicylanilide	68.	3,3',5'-trinitrobenzanilide
34.	5'-methyl-g-salicylanisidide	69.	2',3,4',5'-tetranitrobenzanilide
35.	4'-nitro-g-salicylanisidide	70.	5'-chloro-3,5-dinitro-g-benzanisisidide
		71.	2',5'-diethyl-3,5-dinitrobenzanilide

¹ A.C.S. or I.U.C. nomenclature

(fig. 1 and table 2). 5-nitrosalicylanilides differ with the locus of the nitro group being oriented to the five position. In several instances halogens have also been substituted at the 5 position to produce 5-chloro-3-nitrosalicylanilides or 5-bromo-3-nitrosalicylanilides. Variations in these compounds have been achieved by introducing single (mono) or multiple (poly) substituents in the anilide portion of the molecule. Diversity was attained with isomers by changing the locus of one or all of the substituents. In one case (compound 15, table 2) the 5-substituted nitro group has been reduced to an acetamido group.

RESULTS

NITROSALICYLANILIDES

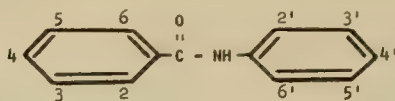
Mono-substituted nitrosalicylanilides.-- This series, with the exception of 2'-ethyl-3-nitrosalicylanilides, 2'-chloro-5-nitrosalicylanilide, and the 5-acetamido- derivative of 3'-chloro-5-nitrosalicylanilide (compounds 3, 11, 15, table 3), effected a complete kill of rainbow trout during a 48-hour

test period at 1 p.p.m. Most of the substituted 3-nitrosalicylanilides at this concentration were lethal within 3 hours. 3-nitrosalicylanilide per se (compound 1, table 3) did not share this property of producing death quickly. Substituted 5-nitrosalicylanilides did not kill fish quickly at less than 10 p.p.m. 4'-phenylazo-3-nitrosalicylanilide and 4'-iodo-3-nitrosalicylanilide (compounds 9 and 10, table 3) were the only mono-halonitrosalicylanilides lethal to trout at 0.1 p.p.m.

Seven of the mono-substituted nitrosalicylanilides (compounds 6-10 and 13-14, table 3) killed all rainbow trout and goldfish at 10 p.p.m. Two of them, 4'-chloro-3-nitrosalicylanilide and 4'-iodo-3-nitrosalicylanilide, were effective within 3 hours. 4'-chloro-3-nitrosalicylanilide was lethal to goldfish at 0.1 p.p.m. but not to rainbow trout during the 48-hour test. Seven others (Nos. 1, 2, 4, 5, 11, 12, and 15) were lethal to trout at 1 p.p.m. but varied in toxicity to goldfish.

The toxicity of mono-substituted nitrosalicylanilides to goldfish increased as the locus of the substituent in the aniline moiety was

TABLE 3.--Relation of chemical structure to piscicidal activity of mono-substituted nitrosalicylanilides to two species of fish at 12°C.



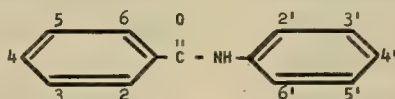
Compound	Locus of substituent on the structure										Toxicity of the compound to--					
											Rainbow trout at--			Goldfish at--		
	2	3	4	5	6	2'	3'	4'	5'	6'	0.1 p.p.m.	1.0 p.p.m.	10.0 p.p.m.	0.1 p.p.m.	1.0 p.p.m.	10.0 p.p.m.
1.	OH	NO ₂	--	--	--	--	--	--	--	--	0	10	¹ 10	0	0	10
2.	OH	NO ₂	--	--	--	I	--	--	--	--	0	10	¹ 10	0	0	¹ 10
3.	OH	NO ₂	--	--	--	C ₂ H ₅	--	--	--	--	0	0	--	0	0	--
4.	OH	NO ₂	--	--	--	--	Cl	--	--	--	0	10	¹ 10	0	6	¹ 10
5.	OH	NO ₂	--	--	--	--	Br	--	--	--	0	¹ 10	¹ 10	0	9	¹ 10
6.	OH	NO ₂	--	--	--	--	I	--	--	--	0	¹ 10	¹ 10	0	10	¹ 10
7.	OH	NO ₂	--	--	--	--	--	Cl	--	--	0	¹ 10	¹ 10	10	¹ 10	¹ 10
8.	OH	NO ₂	--	--	--	--	--	Br	--	--	0	¹ 10	¹ 10	0	10	¹ 10
9.	OH	NO ₂	--	--	--	--	--	I	--	--	9	¹ 10	¹ 10	0	¹ 10	¹ 10
10.	OH	NO ₂	--	--	--	--	--	PA ²	--	--	10	¹ 10	¹ 10	0	10	10
11.	OH	--	--	NO ₂	--	Cl	--	--	--	--	0	8	¹ 10	1	1	¹ 10
12.	OH	--	--	NO ₂	--	--	F	--	--	--	0	10	¹ 10	0	1	10
13.	OH	--	--	NO ₂	--	--	--	Cl	--	--	0	10	¹ 10	1	10	¹ 10
14.	OH	--	--	NO ₂	--	--	--	I	--	--	0	10	¹ 10	1	10	¹ 10
15.	OH	--	--	AcAm ³	--	--	Cl	--	--	--	1	9	¹ 10	0	1	10

¹ All dead at 3 hours.² Phenyl-azo.³ Acetamido.

oriented from ortho (2') to para (4'). For example, although iodo-3-nitrosalicylanilides were evaluated at all three positions, only 2'-iodo-3-nitrosalicylanilide was nontoxic to goldfish at 1 p.p.m. 3'-iodo- and 4'-iodo-3-nitrosalicylanilide are lethal to both species at this concentration, but 4'-iodo-3-nitrosalicylanilide was lethal to both species within 3 hours. In addition the latter compound killed 9 out of 10 rainbow trout at 0.1 p.p.m.,

but the ortho (2') and meta (3') isomers were ineffective. Likewise, 3'-chloro-3-nitrosalicylanilide required 48 hours to kill 6 out of 10 goldfish and all of the rainbow trout at 1 p.p.m. 4'-chloro-3-nitrosalicylanilide produced total kills of both species within 3 hours. Further trials revealed that 4'-chloro-3-nitrosalicylanilide is far more toxic to goldfish than to rainbow trout at 0.1 p.p.m. (tables 4 and 5).

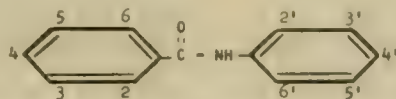
TABLE 4.--Relation of chemical structure of nitrosalicylanilides to selective piscicidal activity for rainbow trout and goldfish at 12°C.



Compound	Locus of substituent on the structure										Toxicity of the compound to--					
											Rainbow trout at--			Goldfish at--		
	2	3	4	5	6	2'	3'	4'	5'	6'	0.1 p.p.m.	1.0 p.p.m.	10.0 p.p.m.	0.1 p.p.m.	1.0 p.p.m.	10.0 p.p.m.
10.	OH	NO ₂	--	--	--	--	--	PA ²	--	--	10	¹ 10	¹ 10	0	10	10
19.	OH	NO ₂	--	Br	--	--	--	Cl	--	--	10	¹ 10	¹ 10	3	10	¹ 10
20.	OH	NO ₂	--	Br	--	--	--	Br	--	--	10	¹ 10	¹ 10	2	10	¹ 10
9.	OH	NO ₂	--	--	--	--	--	I	--	--	9	10	¹ 10	0	10	¹ 10
31.	OH	NO ₂	--	--	--	CH ₃	--	Br	--	--	2	¹ 10	¹ 10	0	10	¹ 10
17.	OH	NO ₂	--	--	--	--	Cl	Cl	--	--	1	¹ 10	¹ 10	1	10	¹ 10
8.	OH	NO ₂	--	--	--	--	--	Br	--	--	0	10	¹ 10	0	¹ 10	¹ 10
6.	OH	NO ₂	--	--	--	--	I	--	--	--	0	¹ 10	¹ 10	0	10	¹ 10
13.	OH	--	--	NO ₂	--	--	--	Cl	--	--	0	10	¹ 10	1	10	¹ 10
14.	OH	--	--	NO ₂	--	--	--	I	--	--	0	10	¹ 10	1	10	¹ 10
33.	OH	NO ₂	--	--	--	CH ₃ O	--	Cl	CH ₃ O	--	0	¹ 10	¹ 10	1	10	¹ 10
7.	OH	NO ₂	--	--	--	--	--	Cl	--	--	0	¹ 10	¹ 10	10	¹ 10	¹ 10

¹ All dead at 3 hours.² C₆H₅-N=N-(phenyl-azo).

TABLE 5.--Obligatory structural requirements for 4'-chloro-3-nitrosalicylanilide as a selective piscicidal agent for goldfish and rainbow trout



Compound	Locus of substituent on the structure										Toxicity of the compound to--					
											Rainbow trout at--			Goldfish at--		
	2	3	4	5	6	2'	3'	4'	5'	6'	0.1 p.p.m.	1.0 p.p.m.	10.0 p.p.m.	0.1 p.p.m.	1.0 p.p.m.	10.0 p.p.m.
7.	OH	NO ₂	--	--	--	--	--	Cl	--	--	0	¹ 10	¹ 10	10	¹ 10	¹ 10
17.	OH	NO ₂	--	--	--	--	Cl	Cl	--	--	1	¹ 10	¹ 10	1	10	¹ 10
39.	OH	NO ₂	--	--	--	--	--	--	--	--	0	0	10	0	0	10
4.	OH	NO ₂	--	--	--	--	Cl	--	--	--	0	10	¹ 10	0	6	10
41.	--	OH	--	--	--	--	Cl	--	--	--	0	0	10	0	0	7
44.	NO ₂	--	--	--	--	--	Cl	--	--	--	0	0	10	0	0	6
50.	--	NO ₂	--	--	--	--	Cl	--	--	--	0	2	¹ 10	0	0	10
53.	--	--	NO ₂	--	--	--	Cl	--	--	--	0	0	0	0	0	0
58.	--	NO ₂	--	NO ₂	--	--	Cl	--	--	--	0	0	7	0	0	0
42.	--	OH	--	--	--	--	--	Cl	--	--	0	0	10	0	0	2
45.	NO ₂	--	--	--	--	--	--	Cl	--	--	0	0	10	0	0	5
54.	--	--	NO ₂	--	--	--	--	Cl	--	--	0	0	10	0	0	6

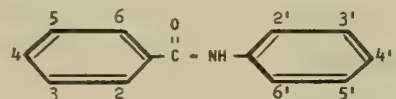
¹ All dead at 3 hours.Poly-substituted nitrosalicylanilides.--

Tests were made to determine whether toxicity and selectivity could be enhanced with poly-substituted derivatives of 3-nitrosalicylanilide. This series includes compounds with multiple substituents, some containing a halogen and a methyl group (CH₃) in the aniline moiety (halo-3-nitrosalicylotoluides),

some with two methyl groups (3'-nitrosalicylotoluides), and others with halogenated-methoxy (CH₃O) derivatives (halo-3-nitrosalicylanilides) (table 6).

3',4'-dichloro-3-nitrosalicylanilide (compound 17, tables 2, 4, and 6) killed all goldfish and rainbow trout at 1 p.p.m., but 9 out

TABLE 6.--Relation of chemical structure to piscicidal activity of poly-substituted nitrosalicylanilides to two species of fish at 12°C.



Compound	Locus of substituent on the structure										Toxicity of the compound to--					
											Rainbow trout at--			Goldfish at--		
	2	3	4	5	6	2'	3'	4'	5'	6'	0.1 p.p.m.	1.0 p.p.m.	10.0 p.p.m.	0.1 p.p.m.	1.0 p.p.m.	10.0 p.p.m.
16.	OH	NO ₂	--	--	--	Cl	--	Cl	--	Cl	0	0	¹ 10	0	0	10
17.	OH	NO ₂	--	--	--	--	Cl	Cl	--	--	1	¹ 10	¹ 10	1	10	¹ 10
18.	OH	NO ₂	--	--	--	Br	--	--	Br	--	0	10	¹ 10	0	3	¹ 10
19.	OH	NO ₂	--	Br	--	--	--	Cl	--	--	10	¹ 10	¹ 10	3	10	¹ 10
20.	OH	NO ₂	--	Br	--	--	--	Br	--	--	10	¹ 10	¹ 10	2	10	¹ 10
21.	OH	NO ₂	--	--	--	CH ₃	CH ₃	--	--	--	0	10	¹ 10	2	0	10
22.	OH	NO ₂	--	--	--	CH ₃	--	CH ₃	--	--	0	10	¹ 10	0	1	¹ 10
23.	OH	NO ₂	--	--	--	CH ₃	--	--	CH ₃	--	0	9	¹ 10	0	0	10
24.	OH	NO ₂	--	--	--	CH ₃	--	--	--	CH ₃	0	1	10	0	0	10
25.	OH	NO ₂	--	--	--	CH ₃	Cl	--	--	--	1	10	¹ 10	0	2	10
26.	OH	NO ₂	--	--	--	CH ₃	--	Cl	--	--	0	¹ 10	¹ 10	0	10	¹ 10
27.	OH	NO ₂	--	--	--	Cl	--	CH ₃	--	--	1	¹ 10	¹ 10	1	¹ 10	¹ 10
28.	OH	NO ₂	--	--	--	CH ₃	--	--	--	Cl	0	0	10	0	0	0
29.	OH	NO ₂	--	--	--	CH ₃	--	--	NO ₂	--	0	0	¹ 10	0	0	9
30.	OH	NO ₂	--	--	--	NO ₂	--	CH ₃	--	--	0	10	¹ 10	0	0	10
31.	OH	NO ₂	--	--	--	CH ₃	--	Br	--	--	2	¹ 10	¹ 10	0	10	¹ 10
32.	OH	NO ₂	--	--	--	CH ₃ O	--	--	Cl	--	0	¹ 10	¹ 10	0	1	¹ 10
33.	OH	NO ₂	--	--	--	CH ₃ O	--	--	CH ₃ O	--	0	¹ 10	¹ 10	1	10	¹ 10
34.	OH	NO ₂	--	--	--	CH ₃ O	--	--	CH ₃	--	0	9	10	1	0	10
35.	OH	NO ₂	--	--	--	CH ₃ O	--	NO ₂	--	--	0	2	¹ 10	1	0	10
36.	OH	NO ₂	--	--	--	NO ₂	--	CH ₃ O	--	--	0	8	¹ 10	0	4	¹ 10
37.	OH	NO ₂	--	--	--	NO ₂	--	--	CH ₃ O	--	0	10	¹ 10	0	0	0
38.	OH	NO ₂	--	--	--	Cl	--	NO ₂	--	--	0	¹ 10	¹ 10	2	10	¹ 10
39.	OH	NO ₂	--	--	--	--	--	--	--	--	0	0	10	0	0	10

¹ All dead at 3 hours.

of 10 of each species survived at 0.1 p.p.m. Compared with 4'-chloro-3-nitrosalicylanilide, this compound was less toxic to goldfish but more toxic to rainbow trout. Also, 2',4',6'-trichloro-3-nitrosalicylanilide (compound 16, table 6) was toxic to goldfish and rainbow trout at 10 p.p.m. but not at 1 p.p.m. It thus appears that the receptors of goldfish, sensitive to 4'-chloro-3-nitrosalicylanilide, cannot easily accommodate bulkier molecules irrespective of a common para (4') substituent. A similar observation on the sea lamprey was made by Starkey and Howell (1965).

A change in locus of the nitro- group from meta (3) to sub-meta (5) on the salicylic acid moiety of para (4') halogenated anilides resulted in slightly more toxicity to goldfish than rainbow trout at 0.1 p.p.m. (compounds 13 and 14, table 6). The toxicity of 5-nitrosalicylanilides to rainbow trout and goldfish was reduced when the halogen was moved to the meta (3') position. The ortho (2') substitution was even less toxic.

Among the poly-substituted 3-nitrosalicylanilides, 4',5-dibromo-3-nitrosalicylanilide and 4'-bromo-5-chloro-3-nitrosalicylanilide (compounds 19-20, tables 4 and 6) were lethal to rainbow trout at 0.1 p.p.m. but only slightly toxic to goldfish. At 1 p.p.m. both compounds were lethal to trout within 3 hours whereas goldfish succumbed later during the 48-hour test.

The toxicity of 3-nitrosalicyloxyllidides (compounds 21-24, table 6) to rainbow trout varies with the loci of the methyl (CH₃) substituents in the aniline moiety. Each compound in this series has an ortho (2') methyl group in common but differs with the ring position of the second substituent either at the meta (3'), para (4'), sub-meta (5'), or sub-ortho (6') positions. The 2',3'- and 2',4'-isomers are completely lethal to rainbow trout at 1 p.p.m. In comparison, the 2',5'- and 2',6'- isomers produce 90- and 10-percent mortality, respectively. None of the isomers was toxic to goldfish except at 10 p.p.m. A similar variation in the toxicity of 3-nitrosalicyloxyllidides of the sea lamprey was reported by Starkey and Howell (1965).

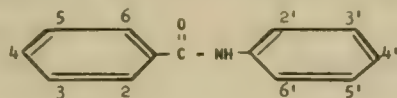
Halo-3-nitrosalicylotoluidides were tested to ascertain if the activity of 3-nitrosalicyloxyllidides is significantly altered by substituting a halogen, nitro, or methoxy group for a methyl group (table 7). 3-nitro-2,3-salicyloxyllidide and 3-nitro-2,4-salicyloxyllidide (compounds 21 and 22) were not toxic to goldfish at 1 p.p.m. Substitution of the meta (3') and para (4') with chlorine atoms, however, produced compounds (3'-chloro-3-nitro-o-salicylotoluidide and 4'-chloro-3-nitro-o-salicylotoluidide, compounds 25 and 26) which were lethal at 1 p.p.m. but not at 0.1 p.p.m. Substitution of the ortho (2') methyl group with a nitro group, as in the case of 2',3-dinitro-p-salicylotoluidide (compound 30, table 7), resulted in a compound which was nontoxic to goldfish at 1 p.p.m.

Other compounds in which one of the methyl groups was replaced by a methoxy group (3-nitrosalicylanisidides) were relatively nontoxic to goldfish at 1 p.p.m. The only exception was 2',3-dinitro-p-salicylanisidide (compound 36) which killed 4 of 10 goldfish. Its isomer, 2',3-dinitro-5-salicylanisidide (compound 37), was nontoxic to goldfish at 10 p.p.m. but killed all rainbow trout at 1 p.p.m. Altering the loci of chloro and methyl groups, as in the case of 2'-chloro-3-nitro-6-salicylanisidide (compound 28) eliminated toxicity to goldfish at 10 p.p.m. In no instance did the halo-3-nitrosalicylotoluidides, 3-nitrosalicyloxyllidides, and 3-nitrosalicylanisidides exhibit selective toxicity to goldfish over rainbow trout. Only one compound, a 2',5'-dimethoxy-3-nitrosalicylanilide, demonstrated a slight toxicity to goldfish at 0.1 p.p.m. and did not affect rainbow trout.

BENZANILIDES

Benzanilides in this series (table 8), differing from salicylanilides by the absence of an ortho hydroxyl substituent, include benzanilide (compound 40), meta (3) hydroxybenzanilides (compounds 41-42), ortho (2) nitrobenzanilides (compounds 43-47), meta (3) nitrobenzanilides (compounds 48-51), para (4) nitrobenzanilides (compounds 52-54), para

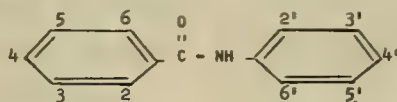
TABLE 7.--Relation of chemical structure to piscicidal activity of 3-nitrosalicyloxylidides, 3-nitrosalicylanisidides, and 3-nitrosalicylotoluidides to two species of fish at 12°C.



	Locus of substituent on the structure										Toxicity of the compound to--					
											Rainbow trout at--			Goldfish at--		
	2	3	4	5	6	2'	3'	4'	5'	6'	0.1 p.p.m.	1.0 p.p.m.	10.0 p.p.m.	0.1 p.p.m.	1.0 p.p.m.	10.0 p.p.m.
21.	OH	NO ₂	--	--	--	CH ₃	CH ₃	--	--	--	0	10	10	2	0	10
25.	OH	NO ₂	--	--	--	CH ₃	Cl	--	--	--	1	10	¹ 10	0	10	¹ 10
22.	OH	NO ₂	--	--	--	CH ₃	--	CH ₃	--	--	0	10	10	0	1	10
26.	OH	NO ₂	--	--	--	CH ₃	--	Cl	--	--	0	¹ 10	¹ 10	1	¹ 10	¹ 10
27.	OH	NO ₂	--	--	--	Cl	--	CH ₃	--	--	1	¹ 10	¹ 10	1	¹ 10	¹ 10
38.	OH	NO ₂	--	--	--	Cl	--	NO ₂	--	--	0	¹ 10	¹ 10	2	10	¹ 10
30.	OH	NO ₂	--	--	--	NO ₂	--	CH ₃	--	--	0	10	¹ 10	0	0	10
36.	OH	NO ₂	--	--	--	NO ₂	--	CH ₃ O	--	--	0	8	¹ 10	0	4	¹ 10
35.	OH	NO ₂	--	--	--	CH ₃ O	--	NO ₂	--	--	0	2	¹ 10	1	0	10
29.	OH	NO ₂	--	--	--	CH ₃	--	--	NO ₂	--	0	0	¹ 10	0	0	9
32.	OH	NO ₂	--	--	--	CH ₃ O	--	--	Cl	--	0	¹ 10	¹ 10	1	10	¹ 10
34.	OH	NO ₂	--	--	--	CH ₃ O	--	--	CH ₃	--	0	9	10	0	0	0
37.	OH	NO ₂	--	--	--	NO ₂	--	--	CH ₃ O	--	0	10	¹ 10	0	0	0
28.	OH	NO ₂	--	--	--	Cl	--	--	--	CH ₃	0	0	10	0	0	0

¹ All dead at 3 hours.

TABLE 8.--Relation of chemical structure to piscicidal activity of mono- and poly-substituted benzanilides to two species of fish at 12°C.



Compound	Locus of substituent on the structure										Toxicity of the compound to--					
											Rainbow trout at--			Goldfish at--		
	2	3	4	5	6	2'	3'	4'	5'	6'	0.1 p.p.m.	1.0 p.p.m.	10.0 p.p.m.	0.1 p.p.m.	1.0 p.p.m.	10.0 p.p.m.
40.	--	--	--	--	--	--	--	--	--	--	0	0	0	0	0	0
41.	--	OH	--	--	--	--	Cl	--	--	--	0	0	10	0	0	7
42.	--	OH	--	--	--	--	--	Cl	--	--	0	0	10	0	0	2
43.	NO ₂	--	--	--	--	Cl	--	--	--	--	0	0	0	0	0	0
44.	NO ₂	--	--	--	--	--	Cl	--	--	--	0	0	10	0	0	6
45.	NO ₂	--	--	--	--	--	--	Cl	--	--	0	0	10	0	0	5
46.	NO ₂	--	--	--	--	--	--	Br	--	--	0	0	10	0	1	2
² 47.	NO ₂	--	--	--	--	CH ₃	Cl	--	--	--	0	0	0	0	0	0
² 48.	--	NO ₂	--	--	--	--	--	--	--	--	0	0	10	0	0	10
49.	--	NO ₂	--	--	--	Cl	--	--	--	--	0	0	10	0	0	1
50.	--	NO ₂	--	--	--	--	Cl	--	--	--	0	2	¹ 10	0	0	¹ 10
² 51.	--	NO ₂	--	--	--	--	Cl	CH ₃	--	--	0	0	0	1	0	0
52.	--	--	NO ₂	--	--	Cl	--	--	--	--	0	0	0	0	0	0
53.	--	--	NO ₂	--	--	--	Cl	--	--	--	0	0	0	0	0	0
54.	--	--	NO ₂	--	--	--	--	Cl	--	--	0	0	10	0	0	6
55.	--	--	Cl	--	--	--	--	--	--	--	0	0	0	0	0	0
56.	--	NO ₂	--	NO ₂	--	--	--	--	--	--	0	0	0	0	0	0
57.	--	NO ₂	--	NO ₂	--	Cl	--	--	--	--	0	0	0	0	0	0
58.	--	NO ₂	--	NO ₂	--	--	Cl	--	--	--	0	0	7	0	0	0
59.	--	NO ₂	--	NO ₂	--	--	Br	--	--	--	0	4	0	0	0	0
60.	--	NO ₂	--	NO ₂	--	--	--	Br	--	--	³ 0	0	0	0	0	0
² 61.	--	NO ₂	--	NO ₂	--	--	--	I	--	--	0	0	0	0	0	0
62.	--	NO ₂	--	NO ₂	--	F	--	--	--	--	0	0	10	0	0	10
63.	--	NO ₂	--	NO ₂	--	CH ₃	--	--	--	--	0	0	10	0	1	8
² 64.	--	NO ₂	--	NO ₂	--	CH ₃	--	CH ₃	--	--	0	0	1	0	0	0
65.	--	NO ₂	--	NO ₂	--	--	Cl	CH ₃	--	--	0	0	2	0	0	0
66.	--	NO ₂	--	NO ₂	--	CH ₃	--	--	Cl	--	0	0	¹ 10	0	0	0
² 67.	--	NO ₂	--	NO ₂	--	CH ₃	Cl	--	--	--	0	0	¹ 10	0	0	0
68.	--	NO ₂	--	NO ₂	--	--	NO ₂	--	--	--	0	0	¹ 10	0	0	0
69.	--	NO ₂	--	NO ₂	--	NO ₂	--	NO ₂	--	--	0	0	10	0	0	0
70.	--	NO ₂	--	NO ₂	--	CH ₃ O	--	--	Cl	--	0	0	0	0	1	0
71.	--	NO ₂	--	NO ₂	--	C ₂ H ₅	--	--	--	C ₂ H ₅	³ 0	³ 0	0	0	0	1

¹ All dead at 3 hours.² Precipitation at 10 p.p.m.³ Sounding.

(4) chlorobenzanilides (compound 55), and 3,5-dinitrobenzanilides (compounds 57-71). As in the substituted salicylanilides, the substituents (mono or poly) are either halogen, methyl, or nitro groups on the anilide portion of the molecule (fig. 1, table 2).

In general, the benzanilides are relatively nontoxic to rainbow trout and goldfish (tables 5 and 8). Only three (compounds 48, 50, and 62) were lethal to goldfish at 10 p.p.m. It appears that substituted benzanilides have minimal toxicity in comparison with salicylanilides. This is attributed to the lack of an ortho hydroxyl substituent. These findings agree with those of Baichwal et al. (1960a) and Starkey and Howell (1965). For example, 3'-chloro-2-nitrobenzanilide and 4'-chloro-2-nitrobenzanilide (compounds 44 and 45, tables 5 and 8) were the most toxic to both species. The speed of activity was increased markedly by moving the nitro group to the meta (3) position in the acid moiety. Substitution of the nitro group at the para (4) position produced compounds intermediate in toxicity.

The 3,5-dinitrobenzanilides were relatively insoluble at 10 p.p.m. and were therefore difficult to bioassay. They were, with several exceptions, selective to rainbow trout at 10 p.p.m. (table 5). Only two (compounds 62 and 63) were significantly toxic to goldfish at this concentration. 3,3',5-trinitrobenzanilide was more toxic than 2',3,4',5-tetranitrobenzanilide (compounds 68 and 69) and may reflect the effects of saturation on both toxicity and selectivity.

Another group of benzanilides (compounds 41, 44, 50, 53, and 58) possess a meta (3') chloro-substituent and vary only in the locus of a substituent in the acid moiety (table 8). Of them, compounds 44, 50, 53, and 58 are various substituted nitrobenzanilides and compound 41 is a substituted 3-hydroxybenzanilide. None has the ortho hydroxyl substituent characteristic of salicylanilides. Compound 53 which has the nitro substituted in the para (4) position was nontoxic to both fishes. The other compounds (41, 44, 50, and 58) were lethal to rainbow trout at 10 p.p.m., but only compound 50 was toxic at 1 p.p.m.

Compounds 41, 44, and 50 varied in their toxicity to goldfish, and only compound 50 killed all specimens at 10 p.p.m. None was toxic to the species at 1 p.p.m. Compound 58 was nontoxic at either concentration.

2',6'-diethyl-3,5-dinitrobenzanilide (compound 71, table 8) was nontoxic to rainbow trout and goldfish but caused sounding reaction among the rainbows at 0.1 and 1 p.p.m.

DISCUSSION

Chemical structures which exhibit the greater toxicity to rainbow trout and goldfish were nitrosalicylanilides. We attribute this to the ortho (2) hydroxy substitution on the acid moiety. Baichwal et al. (1960a) and Starkey and Howell (1965) made similar findings with respect to fungi and sea lampreys respectively. In contrast, benzanilides which do not contain the hydroxy substitution in the ortho position are relatively nontoxic to the test fishes.

The ortho (2) hydroxy substitution also appears to accelerate the biological activity of a compound, because the rainbow trout and goldfish often succumbed in tests within 3 hours.

The substitution of meta (3') nitro on the benzoic acid moiety of benzanilides produced greater toxicity to rainbow trout and goldfish than other nitro substitutions. A nitro substitution in the para (4) position had the least effect of any nitro substitution.

Substitutions of halogens in the para (4') position on the aniline moiety of salicylanilides markedly increased toxicities to fish. They also increase the speed of toxic action. Furthermore, the size of halogen atom has a dramatic effect on selective activity between rainbow trout and goldfish. A para substitution of iodine (atomic weight 127) is most toxic to rainbow trout, whereas bromine (atomic weight 80) is equally toxic to the two species. Chlorine (atomic weight 35) in the para position, however, is selectively toxic to goldfish.

Of the halogen substitutions, those in the ortho (2') position on the aniline moiety of either benzanilides or salicylanilides had least effect. One noteworthy exception occurred when an additional substitution of a nitro was made in the para (4') position. The result was greater toxicity to goldfish over rainbow trout. Similarly, the chloro substitution in the para (4') position in 4'-chloro-3-nitrosalicylanilide caused selective toxicity to goldfish. This particular change in the structural configuration strengthens the concept that selective toxicity occurs when the molecular geometry of a toxicant fits the receptor geometry of the fish. Baichwal et al. (1960b) investigated the fungicidal properties of salicylanilides and derivatives and indicated that activity may be attributed to interference to an enzyme or enzyme system(s) by hydrogen bonding or chelation via the phenol (ortho (4') hydroxy-) and amidic oxygen atoms. Furthermore, according to Ariëns (1964), the introduction of an electron spending group, such as the hydroxy (-OH) in the ortho or para position, increases the electron density in a conjugated system. Conversely, the nitro group decreases the electron density of this system.

Our further substitution of a bromine in the sub-meta (5) position on the salicylic acid moiety of the para (4') halo-3-nitrosalicylanilides gave even greater toxic action to fish in general. Baichwal et al. (1960a) noted a similar increase in toxicity by 5-chloro substitutions on fungicides.

CONCLUSIONS

The chemical structure of salicylanilides and benzanilides was related to toxicity and selectivity to rainbow trout and goldfish. Salicylanilides were more toxic than benzanilides to the fishes. The ortho (2) hydroxy substitution of salicylanilide accelerates biological activity against fish. The meta (3) nitro substitution on the salicylanilides and benzanilides increases toxicity to fish. The para (4') halogen substitution on nitrosalicylanilides markedly increased toxicities to fish and enhanced the speed of toxic action. The

size of the halogen atom substituted on 3-nitrosalicylanilide affected selective toxicity between rainbow trout and goldfish. The ortho (2') halogen substitution on 3-nitrosalicylanilide became selectively toxic to goldfish by adding a para (4') nitro on the aniline moiety. The para (4') halogen substitution on 5-nitrosalicylanilides caused slight selective toxicity to goldfish. A para (4') chloro substitution on the 2',5'-dimethoxy-3-nitrosalicylanilide resulted in selective toxicity to goldfish. A bromine substituted in the sub-meta (5) position on para (4') halo-3-nitrosalicylanilides increased toxicity to fish.

SUMMARY

Relations between the chemical structures of salicylanilides and benzanilides and their toxicity to rainbow trout and goldfish were investigated. Single and multiple substitutions of alkyl, nitro, and halo groups on the salicylanilides and benzanilides were tested in standard, static bioassays with fingerling fish at the Fish Control Laboratory.

The nitrosalicylanilides were more toxic to the fish than benzanilides. A hydroxy substitution at ortho (2) appears to accelerate toxic action. The nitro substitution at meta (3) or sub-meta (5) on the benzoic acid moiety caused greater toxicity to the fish than other nitro substitutions.

Halogens in the para (4') position on the aniline moiety of salicylanilides markedly increased toxicities to fish and accelerated toxic action. The size of the halogen atom was found to have a great effect on selective activity between the two fishes. Iodine in the para (4') position produced greater toxicity to rainbow trout, whereas chlorine in the same position brought about an increase in toxicity to goldfish.

Halogens in the meta position were less toxic than para, and least toxic in the ortho position. An exception occurs when the chloro in the ortho (2') position was accompanied by a nitro in the para (4') position. The resulting compound was selectively toxic to goldfish. Halogenation in the sub-meta (5) position on the salicylic acid moiety produced even greater toxicity to both species.

The results of changes in structural configuration of salicylanilides support the concept that general and selective toxicity occurs when molecular geometry of a toxicant conforms to the receptor geometry of the fish.

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10. Evaluation of p,p'-DDT as a Reference Toxicant in Bioassays

By Leif L. Marking, Chemist
Fish Control Laboratory
Bureau of Sport Fisheries and Wildlife
La Crosse, Wisconsin



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EVALUATION OF p,p'-DDT AS A REFERENCE TOXICANT IN BIOASSAYS

By Leif L. Marking, Chemist
Fish Control Laboratory, La Crosse, Wis.

ABSTRACT.--p,p'-DDT was tested as a reference standard toxicant against 19 species of freshwater fish, including 39 lots from 10 sources. In particular, the rapidity, nonselectivity, and consistency of its toxicity to fish were evaluated in 96-hour static bioassays. The chemical was rapidly and consistently toxic to lake trout, carp, green sunfish, bluegill, and yellow perch. It lacked either rapid or consistent toxicity to rainbow trout, brook trout, goldfish, fathead minnows, and longear sunfish in 96-hour tests. Thus, p,p'-DDT is of limited usefulness as a reference standard toxicant in large bioassays with many species of fish.

Necessity for standardization of facilities and techniques in bioassay, and reproducibility and comparability of results from test to test and investigation to investigation was recognized early in the fish control program. Others have also emphasized this necessity. Hart, Doudoroff, and Greenbank (1945) stated the need for a reference evaluation in bioassays to serve as a link between work of different investigators and to compare the relative toxicities of substances. Warren and Doudoroff (1958) observed that living organisms are highly variable and in order to obtain reproducible results in bioassays, uniformly tolerant test organisms must be found. Cutting et al. (1959) pointed out the unique advantages of fish as indicator organisms, but noted the persistent difficulties in making needed comparisons of bioassays because of the variety of fishes used. Cope (1961) suggested that it is essential to have some measure of the condition of test fish which are used in bioassays. Douglas and Irwin (1962) stressed the fact that results of independent bioassays often cannot be related because the comparative resistance of many test fishes has not been established. They pointed out the need for knowledge on the reactions of different species of fish when exposed to a particular toxicant.

Standardization of bioassay facilities and methods at the Fish Control Laboratories,

La Crosse, Wis., and Warm Springs, Ga., were reported by Lennon and Walker (1964). The reproducibility and comparability of results in bioassays may be achieved best by employing a standardized test fish, but there is none at present. In lieu of a standard test fish, the potentials of p,p'-DDT as a standard reference chemical in bioassays were investigated.

A standard reference chemical should have the following qualifications:

1. A rapid, nonselective, and consistent toxicity to fish.
2. Uncommon in nature, thus reducing risks of previous exposure among fish.
3. A known mode of action on fish.

The purpose of this study was to determine whether p,p'-DDT has the first qualification. Accordingly, we evaluated the toxicity of the chemical against 19 species of freshwater fish, including 39 separate lots of fish from 10 different sources.

METHODS AND MATERIALS

The bioassays of p,p'-DDT [1,1,1-trichloro-2,2-bis (p-chlorophenyl) ethanol] at La Crosse and Warm Springs were static tests

in 5-gallon glass jars as described by Lennon and Walker (1964). Stock solutions of the reagent grade chemical were made in acetone, and aliquots were added directly to bioassay vessels. The quantity of acetone in bioassays never exceeded 1 ml. per l. The 96-hour LC₅₀ values (concentrations producing 50-percent mortality) were calculated according to the method of Litchfield and Wilcoxon (1949). This method gives a 95-percent confidence interval, the range in which 50 percent of the fish die 95 percent of the time.

The species and sources of fish are listed in table 1. Most of the lots were received from National Fish Hatcheries, but one lot of brook trout was received from a Wisconsin State Hatchery and two species were collected at the Necedah National Wildlife Refuge in Wisconsin. A "lot" is a particular group of fish of a species received from a hatchery in a single shipment.

TABLE 1.--Species and source of fish used in bioassays of p,p'-DDT

Common name	Technical name	Source
Rainbow trout.....	<u>Salmo gairdneri</u>	Manchester NFH, Ia.
Brown trout.....	<u>Salmo trutta</u>	Manchester NFH, Ia.
Brook trout.....	<u>Salvelinus fontinalis</u>	Lake Mills NFH, Wis.
Brook trout.....	<u>Salvelinus fontinalis</u>	St. Croix Falls SFH, Wis.
Lake trout.....	<u>Salvelinus namaycush</u>	Jordan River NFH, Mich.
Northern pike.....	<u>Esox lucius</u>	Yankton NFH, S.D.
Goldfish.....	<u>Carassius auratus</u>	Lake Mills NFH, Wis.
Northern redbelly dace.....	<u>Chrosomus eos</u>	Necedah NWR, Wis.
Carp.....	<u>Cyprinus carpio</u>	Lake Mills NFH, Wis.
Fathead minnow.....	<u>Pimephales promelas</u>	Lake Mills NFH, Wis.
Black bullhead.....	<u>Ictalurus melas</u>	Guttenberg NFH, Ia.
Channel catfish....	<u>Ictalurus punctatus</u>	Fairport NFH, Ia.
Brook stickleback..	<u>Eucalia inconstans</u>	Necedah NWR, Wis.
Green sunfish.....	<u>Lepomis cyanellus</u>	Lake Mills NFH, Wis.
Pumpkinseed.....	<u>Lepomis gibbosus</u>	Lake Mills NFH, Wis.
Bluegill.....	<u>Lepomis macrochirus</u>	Lake Mills NFH, Wis.
Bluegill.....	<u>Lepomis macrochirus</u>	Warm Springs NFH, Ga.
Longear sunfish....	<u>Lepomis megalotis</u>	Fairport NFH, Ia.
Largemouth bass....	<u>Micropterus salmoides</u>	Cenosa NFH, Wis.
Yellow perch.....	<u>Perca flavescens</u>	Lake Mills NFH, Wis.
Fresh water drum...	<u>Aplodinotus grunniens</u>	Lake Mills NFH, Wis.

Upon delivery, the fish were maintained in a fish holding house and fed commercial pellets. The lengths and weights of representative samples in each lot were measured routinely. The fish at La Crosse and Warm Springs were held and tested at 12° and 17° C., respectively. Before testing, each lot was in quarantine for 10 days; food was withheld the last 3 days. If, after quarantine, a lot of fish was judged acceptable for bioassay, it was used in tests of p,p'-DDT and candidate fish controls. The tests with p,p'-DDT were

repeated biweekly for as long as a lot remained on hand in usable condition. Subsequent acquisitions of the same species were assigned different lot numbers even though they may have come from the same hatchery stock as a previous lot.

Mortalities of the test fish were recorded throughout each 96-hour bioassay. Preliminary investigations disclosed that p,p'-DDT produces similar effects on all species of fish, although there are slight variations in the behavior of different species. Reactions in catfishes, for instance, are less obvious than in trout. Generally, irritation and excitation are readily noticeable in the earlier stages of response. Swimming becomes erratic, upside down, on a side, with surfacing and colliding with walls of the container. The swimming is continuous until the final stages of response. Total loss of equilibrium, loss of reflex reactivity, and cessation of locomotion immediately precede death. Moribund fish often turn light in color. The operculums are usually distended and the spine is sometimes curved, indicating convulsive reactions at the time of death.

RESULTS

The toxicity of p,p'-DDT differed greatly among the 19 species of fish. The compound was consistently very toxic to some fishes but less and inconsistently so to others (table 2). The toxicity also varied among lots of a species, and details are given in tables 3 to 7.

TABLE 2.--Order of toxicity of p,p'-DDT to various species of fish

Species	Number of lots	Number of tests	Mean LC ₅₀ in p.p.b.
Largemouth bass.....	1	1	0.8
Yellow perch.....	2	3	0.9
Northern pike.....	1	1	1.7
Green sunfish.....	3	8	4.5
Pumpkinseed.....	2	5	4.5
Bluegill.....	8	10	4.5
Carp.....	4	6	8.2
Longear sunfish.....	1	2	8.7
Lake trout.....	1	2	9.3
Freshwater drum.....	1	1	10.0
Rainbow trout.....	4	8	10.7
Brown trout.....	1	1	10.9
Brook trout.....	2	4	11.5
Channel catfish.....	1	2	17.5
Black bullhead.....	1	4	25.8
Goldfish.....	4	7	58.7
Brook stickleback.....	1	1	67.0
Northern redbelly dace.....	1	1	68.0

TABLE 3.--Toxicity of p,p'-DDT to trout and northern pike in 96 hours at 12°C.

Species and lot	Average weight (grams)	Date tested	LC ₅₀ (p.p.b.)	95-percent confidence interval
Rainbow trout:				
Lot 16a.....	0.9	3-24-64	14.0	12.8-15.4
Lot 17.....	0.9	4-10-64	4.6	2.9- 7.4
Do.....	1.2	4-24-64	7.2	6.0- 8.7
Lot 18.....	1.6	5-15-64	15.0	12.0-19.0
Do.....	1.9	5-22-64	17.0	14.4-20.1
Do.....	2.5	6- 5-64	13.0	9.7-17.4
Do.....	3.1	6-19-64	12.0	10.7-13.4
Lot 159.....	0.5	2- 5-65	2.4	1.7- 3.3
Brown trout: Lot 26.....	4.0	6- 5-64	10.9	9.6-12.3
Brook trout:				
Lot 18.....	1.4	4-10-64	7.2	5.2-10.1
Do.....	3.3	4-24-64	17.0	14.3-20.2
Do.....	3.3	5- 1-64	20.0	11.9-33.6
Lot 161.....	0.4	2- 5-65	1.8	1.2- 2.4
Lake trout:				
Lot 78.....	2.5	8-21-64	9.1	8.0-10.4
Do.....	2.8	9-18-64	9.5	8.5-10.6
Northern pike: Lot 34.....	0.5	5-30-64	1.7	1.4- 2.0

TABLE 6.--Toxicity of p,p'-DDT to centrarchids in 96 hours at 12°C.

Species and lot	Average weight (grams)	Date tested	LC ₅₀ (p.p.b.)	95-percent confidence interval
Green sunfish:				
Lot 25.....	0.7	5-22-64	2.8	2.3- 3.4
Do.....	1.0	6- 5-64	3.0	2.5- 3.6
Do.....	1.4	6-20-64	3.9	3.1- 4.9
Lot 95.....	1.1	10- 9-64	6.7	5.0- 9.0
Do.....	0.8	10-29-64	6.4	5.6- 7.3
Do.....	0.7	11-20-64	4.4	3.6- 5.4
Lot 145.....	0.8	12-31-64	3.6	2.7- 4.8
Do.....	0.8	1-22-65	5.0	4.1- 6.1
Pumpkinseed:				
Lot 118.....	1.4	10-29-64	7.5	6.4- 8.8
Do.....	1.4	11-13-64	6.7	5.7- 7.8
Lot 147.....	1.3	12-11-64	2.8	2.3- 3.4
Do.....	1.3	12-31-64	3.6	2.7- 4.8
Do.....	1.3	1- 8-65	1.8	1.2- 2.6
Bluegill:				
Lot 22.....	0.6	4-24-64	4.3	3.5- 5.3
Do.....	0.6	5- 8-64	1.7	1.3- 2.1
Lot 96.....	1.1	9-18-64	3.0	2.3- 4.0
Lot 112.....	1.3	10-16-64	7.0	6.6- 7.4
Lot 115.....	0.6	10-29-64	7.0	6.4- 7.7
Lot 131.....	1.1	12- 4-64	3.6	2.6- 5.0
Lot 152.....	0.8	1- 5-65	1.2	0.9- 1.6
Lot W32.....	0.9	12- 3-64	4.6	3.7- 5.8
Do.....	1.2	12-14-64	9.4	6.4-13.9
Do.....	1.0	12-28-64	2.8	1.9- 4.0
Longear sunfish:				
Lot 123a.....	1.0	11-20-64	4.9	4.0- 6.0
Do.....	1.0	12- 4-64	12.5	7.6-20.6
Largemouth bass: Lot 57....	0.5	7-17-64	0.8	0.7- 0.9

TABLE 4.--Toxicity of p,p'-DDT to cyprinids in 96 hours at 12°C.

Species and lot	Average weight (grams)	Date tested	LC ₅₀ (p.p.b.)	95-percent confidence interval
Goldfish:				
Lot 1.....	2.5	3-4-64	76.0	27.0-213.0
Lot 19.....	2.4	3-31-64	27.0	15.0- 50.0
Do.....	2.4	4- 3-64	32.0	17.0- 61.0
Lot 75.....	1.9	8-11-64	180.0	125.9-257.4
Lot 119.....	0.8	11-13-64	40.0	22.8- 70.0
Do.....	0.7	11-27-64	35.0	25.9- 47.2
Do.....	1.0	12-11-64	21.0	13.1- 33.6
Northern redbelly dace:				
Lot 154.....	1.1	1- 8-65	68.0	41.2-112.2
Carp:				
Lot 74.....	2.2	9- 4-64	9.2	4.6- 18.4
Lot 83.....	2.0	9-11-64	4.0	1.3- 12.0
Do.....	2.1	9-25-64	11.3	8.7- 14.7
Do.....	2.5	10- 9-64	12.0	6.0- 24.0
Lot 148.....	0.8	1- 8-65	6.9	5.1- 9.3
Lot 157.....	0.6	1-22-65	6.0	4.7- 7.6

TABLE 5.--The toxicity of p,p'-DDT to catfishes and brook stickleback in 96 hours at 12°C.

Species and lot	Average weight (grams)	Date tested	LC ₅₀ (p.p.b.)	95-percent confidence interval
Black bullhead:				
Lot 107.....	2.1	10- 9-64	42.0	33.6-52.5
Do.....	2.4	10-30-64	23.5	16.1-34.3
Do.....	2.3	11-13-64	17.0	12.6-23.0
Do.....	2.2	11-27-64	20.0	13.3-30.0
Channel catfish:				
Lot 92.....	1.9	9- 4-64	17.5	8.3-36.8
Do.....	2.0	9-18-64	17.5	10.3-29.8
Brook stickleback:				
Lot 127.....	1.6	12- 4-64	67.0	54.5-82.4

TABLE 7.--Toxicity of p,p'-DDT to yellow perch and freshwater drum in 96 hours at 12°C.

Species and lot	Average weight (grams)	Date tested	LC ₅₀ (p.p.b.)	95-percent confidence interval
Yellow perch:				
Lot 116.....	1.1	11-13-64	0.8	0.6- 1.0
Do.....	0.8	11-27-64	0.6	0.4- 0.8
Lot 151.....	1.2	1-22-65	1.5	1.1- 2.0
Freshwater drum: Lot 117...	3.3	10-30-64	10.0	5.6-18.0

The p,p'-DDT was most toxic to largemouth bass, yellow perch, and northern pike, in that order; mean LC₅₀ values were 0.8, 0.9, and 1.7 p.p.b. The fish were small fingerlings, however, and they did not appear to hold up well in the 2 to 4 days of starvation during the test period. Had live food been available in quantity before and during the tests, the fish might have shown greater resistance to the toxicant. Furthermore, the holding and test temperature of 12° C. may have been below optimum for fingerlings of these species.

The mean LC₅₀ of the toxicant was 4.5 p.p.b. for green sunfish, pumpkinseed, and bluegill (table 2). The toxicity ranged from 2.8 to 6.7 p.p.b. for green sunfish, 1.8 to 7.5 p.p.b. for pumpkinseed, and 1.2 to 9.4 p.p.b. for

bluegill, and the higher values for them occurred in October (table 6). Green sunfish in lot 25 doubled their average weight, 0.7 to 1.4 g. in a month, and the LC_{50} increased from 2.8 to 3.9 p.p.b. In contrast, green sunfish in lot 95 lost in average weight from 1.1 to 0.7 g. and LC_{50} from 6.7 to 4.4 p.p.b. The LC_{50} values for lots of the three species which merely maintained their average weights over 2-week to 1-month periods varied up and down, but the trend was generally downward.

The p,p' -DDT produced consistent results among bluegills tested at La Crosse and Warm Springs. The biweekly LC_{50} values for one lot of longear sunfish increased from 4.9 to 12.5 p.p.b. Since the confidence intervals did not overlap, the difference was significant (table 6).

The chemical was less toxic to carp than to most of the centrarchids but more toxic than to trouts (table 2). It was tested three times against carp in lot 83 which fed, grew fairly rapidly, and became more resistant.

The toxicity of the compound was intermediate to trouts (table 2). Of the four species, it was most toxic to lake trout and least to brook trout. The data suggest selectivity to some degree among these closely related species. Hatch (1957) related an interesting example of different responses among salmonids to DDT. An aerial application of the insecticide to a hatchery watershed was sublethal to rainbow and brook trout but killed all landlocked Atlantic salmon. His further observations confirmed that the trouts could survive concentrations deadly to the salmon.

The bioassays included four lots of rainbow trout, and the LC_{50} values for them were inconsistent. Generally, the larger fish were more resistant to the chemical. The mean LC_{50} for trout over 1.5 g. was approximately 14. p.p.b. while the value dropped to 7 p.p.b. for fish less than this weight.

The candidate chemical was less toxic to channel catfish and black bullhead (table 2). There was only one lot of channel catfish available, however, and the fish showed identical tolerances in tests 2 weeks apart. The

LC_{50} for black bullhead declined from 42 to 17 p.p.b. over a 7-week period (table 5).

The p,p' -DDT was one-fifth as toxic to goldfish as to trouts. Moreover, the interlot and intralot differences in responses to the toxicant were great. The LC_{50} values for lots ranged from 21 to 180 p.p.b., and confidence intervals were extremely wide (table 4). These intervals do not overlap in all instances and suggest that the tolerance differs.

The p,p' -DDT was least toxic to brook stickleback (table 5) and northern redbelly dace (table 4), and their resistances exceeded the mean tolerance of goldfish. The LC_{50} values are six times as great as the mean for trouts (table 2).

Tests of the toxicant against fathead minnows presented unique problems. The results were neither rapid nor consistent. The toxicity did not increase uniformly with increased concentrations up to 1,000 p.p.b. within 96 hours. A reliable LC_{50} could not be obtained in repeated trials. Furthermore, a marked abdominal distention occurred in exposed fish. It began at 24 hours and became more pronounced at 96 hours. Examination of the peritoneal cavity revealed an enlarged air bladder. It is not known at present what relation this distention has to the toxicant. Henderson, Pickering, and Tarzwell (1959) noted the phenomenon among fathead minnows which had been exposed to chlorinated hydrocarbons. They indicated, however, that the LC_{50} of p,p' -DDT to fatheads was 32 p.p.b. in 24 hours and 26 p.p.b. in 96 hours. We could not confirm their results.

DISCUSSION

The p,p' -DDT was rapidly and consistently toxic to lake trout, carp, channel catfish, green sunfish, bluegill and yellow perch, but not so to rainbow trout, brook trout, and black bullhead. Results were unsatisfactory with goldfish and fathead minnows in 96-hour bioassays. The regression curves, plotted on logarithmic coordinates, in figure 1 illustrate the consistency of toxic responses in rainbow trout and goldfish. The calculated slope function

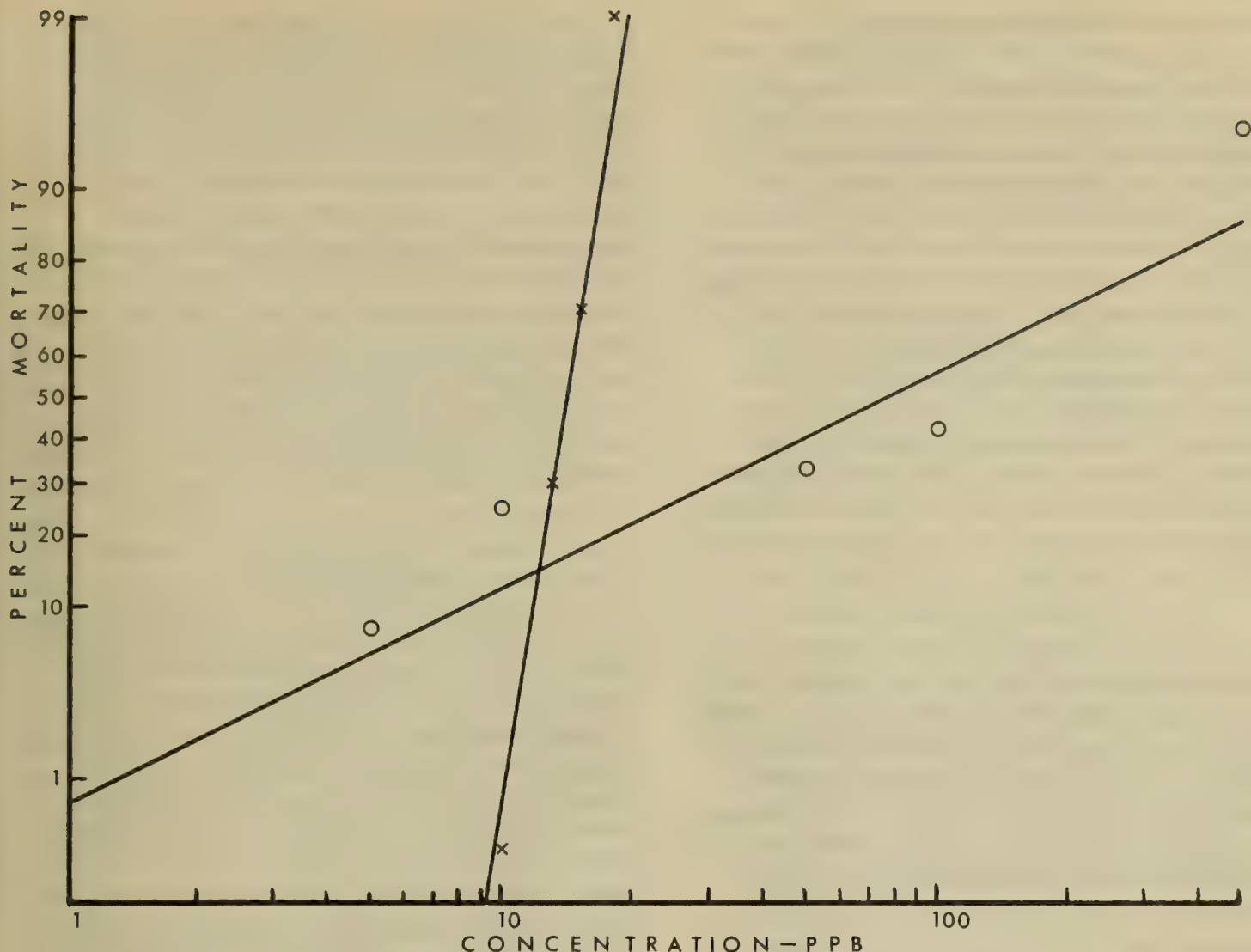


Figure 1. --Responses of rainbow trout (x) and goldfish (o) to p,p' -DDT.

for rainbow trout is 1.16 whereas that for goldfish is 6.02.

The regression curve for rainbow trout (lot 16a) indicates toxicity at very low levels. The slope of the curve reveals a narrow margin between survival and mortality. The confidence intervals are relatively narrow, thus indicating that toxicity is more accurately defined.

The regression curve for goldfish (lot 1) contrasts sharply with that for rainbow trout. The curve is flat rather than steep, and it shows that increased concentrations in the survival and mortality range produce little effect within a 96-hour bioassay. The confidence intervals are wide, and the toxicity of p,p' -DDT to goldfish is not defined accurately.

The results with p,p' -DDT further demonstrated that lots of fish within a single species may vary significantly in responses. The variations may be due to several factors. Centrarchids appeared to have seasonal cycles in relative resistance to the toxicant. Green sunfish, pumpkinseed, and bluegill displayed higher tolerances in autumn (table 6). It was impossible, however, to evaluate total cyclic effects since the fish were not available for more than a few months of the year. Shell (1961) found systematic and seasonal variations in blood components of smallmouth bass. He noted that most of the changes took place in August and suggested that changes in the anabolic-catabolic balance were responsible for the cyclic concentrations of the various blood components. This may explain why fish are at times more vulnerable to toxicants or other stresses.

Holden (1962) determined that fish in poor condition or with a low reserve of fatty tissue are more sensitive to DDT. This was noticeable in some of our tests with pumpkinseed, bluegill, and yellow perch. In contrast, relatively healthy fish which were feeding and gaining in weight tended to become more resistant to the toxicant. Examples of increasing resistance were afforded by rainbow trout (lot 17), brook trout (lot 18), goldfish (lot 19), carp (lot 83), and green sunfish (lot 25). Phillips, Livingston, and Dumas (1960) reported that starved brook trout have a tendency to increase in water content and decrease in body fat and protein. Such changes in body chemistry may contribute to decreased resistances of the fish to the reference compound.

The bioassays of p,p' -DDT showed that fish obtained from different sources display different tolerances. Geographic location, water quality, pond fertilization, herbicide applications, and feeding and handling during the rearing period probably influence the relative tolerance of specimens to a toxicant. Lots of brook trout from Lake Mills National Fish Hatchery and St. Croix Falls State Fish Hatchery varied considerably in resistance. On the other hand, the bluegills from Lake Mills and Warm Springs National Fish Hatcheries reacted quite similarly.

A good reference standard chemical should be sufficiently uncommon in nature that there is little risk that bioassay fishes have been exposed to it or its closely related compounds. The p,p' -DDT hardly meets this qualification, since bioassay fishes from various hatcheries may have been exposed previously to DDT. It is very widely used as a pesticide and resists decomposition. A hatchery in an agricultural watershed, for example, may very well be contaminated to some extent. Also, the natural or prepared diets of the fish are likely to contain small quantities of DDT. Such exposures to the insecticide may have influenced the tolerances of fish in our tests. Many of the trials were unsuccessful because all specimens either lived or died at concentrations of p,p' -DDT which had previously resulted in partial kills.

Cope (1960) reported that trout and whitefish retained DDT in considerable quantities almost a year after an aerial application for spruce budworm in a western watershed. King (1962) found that guppies became more tolerant to DDT in repeated exposures. Vinson, Boyd, and Ferguson (1963) observed that mosquitofish (*Gambusia affinis*) preexposed to DDT near treated cotton fields were considerably more tolerant than fish from untreated areas. Boyd and Ferguson (1964) observed that tolerance to DDT persisted through one to three generations of mosquitofish which were removed from an environment contaminated by the insecticide.

CONCLUSIONS

The p,p' -DDT partially fulfilled the qualification that a reference standard toxicant be rapidly, nonselectively, and consistently toxic. It was rapidly and consistently toxic to lake trout, carp, channel catfish, green sunfish, bluegill, and yellow perch. It was sufficiently toxic to brown trout, northern pike, northern redbelly dace, black bullhead, brook stickleback, largemouth bass, and freshwater drum, but there were not enough lots of each species to determine the consistence of toxicity. It lacked either rapid toxicity or consistent toxicity to rainbow trout, brook trout, goldfish, fathead minnows, and longear sunfish in 96-hour bioassays. The p,p' -DDT exhibited a selective toxicity among the 19 fishes instead of the desired nonselectivity. For example, the mean LC_{50} for largemouth bass was 0.8 p.p.b. whereas the LC_{50} for northern redbelly dace was 68 p.p.b. Moreover, a reliable LC_{50} for fathead minnows could not be obtained within a 96-hour bioassay.

There were intraspecific as well as interspecific differences in the sensitivity of fish to the toxicant. For example, the range of LC_{50} values for four lots of goldfish was 21 to 180 p.p.b. The p,p' -DDT was generally more toxic to small fish than large ones within a species. Thus, p,p' -DDT is limited in usefulness as a reference standard in large-scale bioassays with many species of freshwater fish. The tests against 19 species demonstrate the continuing need for an adequate

standard reference compound and/or a standard reference fish. In spite of the limitations, it is useful in the selection, testing, and evaluation of other candidate reference compounds.

SUMMARY

A standard reference compound is needed in the fish control program to facilitate reproducibility and comparisons in bioassays in which many species, sizes, conditions, and sources of fish, and many kinds and concentrations of chemicals are involved. The p-p'-DDT was tested for rapid, nonselective, and consistent toxicity against 19 fishes, including different lots of the same species and repeated samples within lots. The trials were accomplished in static bioassays in the laboratory at 12° C. We established the LC_{50} of p,p'-DDT for each lot of fish received from hatcheries or the field, but only after 10 days of quarantine in a fish-holding house. The tests were repeated biweekly for as long as a lot remained on hand in testworthy condition.

The p,p'-DDT was rapidly and consistently toxic in 96-hour bioassays to 6 of the 19 species. It was sufficiently toxic to 8 others, but there were insufficient lots to evaluate consistency. It lacked either rapid toxicity or consistent toxicity to five species. The mean LC_{50} for largemouth bass was 0.8 p.p.b., and the mean for northern redbelly dace was 68 p.p.b., thus it was more selective in its action than would be desirable.

There were intraspecific as well as interspecific differences in sensitivity among the fish to the toxicant. The chemical was more toxic to small fish than large ones within a species.

Since the toxicity of p,p'-DDT varied in rapidity, selectivity, and consistency against 19 species of freshwater fish, the compound is of limited usefulness as a standard reference in large-scale bioassays. The results are helpful, however, in the selection, testing, and evaluation of other candidate reference compounds.

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11. Evaluation of an Electronic Method of Measuring Hematocrits of Fish

By Richard A. Schoettger and Arnold M. Julin, Fishery Biologists
Fish Control Laboratory
Bureau of Sport Fisheries and Wildlife
La Crosse, Wisconsin



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EVALUATION OF AN ELECTRONIC METHOD OF MEASURING HEMATOCRITS OF FISH

By Richard A. Schoettger and Arnold M. Julin, Fishery Biologists
Fish Control Laboratory, La Crosse, Wis.

ABSTRACT.--Conductivity measurements of mammalian blood are considered satisfactory estimates of hematocrit. The YSI Electronic Hematocrit gives rapid and reproducible results with fish blood, and the readings can be corrected to microhematocrit values. In the low hematocrit range, however, readings do not change proportionately with microhematocrits; they are difficult to correct; but they may prove useful as a gross, rapid method for detecting anemia. Electrolyte and protein concentrations in fish blood influence the electronic hematocrit; the relative size of erythrocytes may also.

Hematological parameters are used frequently to estimate the physiological condition of fish. Among them, the hematocrit is one of the easier values to secure. Procedures, problems, and modifications associated with microhematocrit determinations in fish were discussed by Hesser (1960), Snieszko (1960), Larsen and Snieszko (1961), and Mairs and Kennedy (1962).

The microhematocrit, or centrifuge, technique is better suited to laboratory routine than to measurements of hematocrit in the field. Electric line power is needed for the centrifuge, and transportation of blood samples to the laboratory is time consuming. There is need for a method which complements the centrifuge technique in the laboratory and is convenient for studies in the field.

An electrical device for relating the conductivity of mammalian blood to the hematocrit was described by Okada and Schwan (1960). They pointed out that its validity is affected by changes in serum conductivity or the average size of erythrocytes. Kern et al. (1961) investigated the reproducibility of results obtained with the instrument on human blood and studied the effect of anticoagulants

and blood protein levels on conductivity. Further tests were conducted by the Yellow Springs Instrument Company, which manufactures a similar instrument called an Electronic Hematocrit.

Our investigation was designed to study the comparability of the YSI electronic and centrifuge methods for measuring hematocrits in fish, the reproducibility of the electronic hematocrits, and some physiological variables in the blood of fish which could influence conductivity.

METHODS AND MATERIALS

Hematocrits were determined as described by Larsen and Snieszko (1961) for the centrifuge method and according to the manufacturer's instructions for the electronic method.

Briefly, the YSI Model 30 Electronic Hematocrit is portable, weighs 2.8 pounds, is powered by a 6-volt mercury cell, and is compensated for use at 50° to 105° F. A blood sample of at least 0.02 cc. is drawn and placed between two electrodes imbedded

in a glass cell. The cell is placed between the circuit contacts, a button is pressed, and the hematocrit is read directly from the instrument. The reproducibility of readings is reported by the manufacturer to be 0.1 unit with a standard deviation of ± 0.3 unit. Anticoagulants other than heparin and abnormal levels of serum proteins in human blood influence conductivity.

The operating principle of the electronic hematocrit is based on the insulating characteristics of the erythrocyte membrane which separates the conductive interior from the conductive serum outside the cell. The YSI instrument contains a bridge circuit. Two arms of the bridge consist of a precision, center-tapped transformer. The third is a thermistor-resistor which automatically compensates for ambient and glass cell temperatures. A fixed volume of blood within a glass cell constitutes the unknown fourth resistance. Unbalance of the bridge is a measure of the concentration of erythrocytes (manufacturer's specifications; and Okada and Schwan, 1960).

Blood samples were collected from fish by cardiac puncture with a syringe or from severed caudal vessels directly into capillary tubes. The syringes, tubes, and scalpels were coated with heparin.

The reproducibility of the two hematocrit methods was estimated by repeated measurements on relatively large samples of blood. The samples were secured from one each shovelnose sturgeon, spotted sucker, northern redhorse, brown bullhead, white bass, largemouth bass, and walleye. During the measurement of hematocrits a magnetic mixer was used to maintain uniform distribution of red blood cells within a sample, as described by Snieszko (1963).

Tests of the comparability of the hematocrit methods were conducted with fingerling and larger-size fish including: rainbow trout, brown trout, brook trout, lake trout, and goldfish. Two capillary tubes were filled

from each specimen. One was spun in an International Microcapillary Centrifuge, Model MB, and the hematocrit determined with a plastic reader. The second sample was utilized for electrical measurements.

The effects of sodium chloride and protein concentrations on electronic hematocrits of rainbow trout and carp were determined in an experimental design similar to that of Kernen et al. (1961) except that the tests were conducted with salines containing 0.65-, 0.75-, 0.85-, and 1.0-percent sodium chloride. According to Wolf (1963) this range of salines has been used in physiological studies of fish. Powdered bovine albumin was added to the salines to obtain protein levels of 1 to 9 grams per 100 cc. This albumin was selected because it was used by Kernen et al. (1961). The erythrocytes were separated from heparinized blood by centrifuging at 850 r.p.m. for 15 minutes. The cells were washed twice in the appropriate saline at 20° C. and resuspended in various protein-saline solutions to give approximate hematocrits of 25 to 30 percent. A third saline rinse occasionally resulted in hemolysis of the erythrocytes in the final resuspension. Wolf (1959) found that red cells of salmonids coagulated in various sodium chloride solutions which did not contain anticoagulant. In our tests, residual heparin probably served a similar function. Ionic coagulants such as the oxalates alter the conductivity of samples (Kernen et al., 1961).

Throughout the tests, capillary tubes were examined for clotting and hemolysis. The electronic cells were checked for clots. It was essential to conduct electrical measurements rapidly to minimize the effect of erythrocyte sedimentation within the glass cell.

The trout and goldfish were obtained from State and National fish hatcheries. The other species were collected in the Mississippi River. They were maintained in flowing well water at the Fish Control Laboratory for at least 2 months before use.

RESULTS AND DISCUSSION

REPRODUCIBILITY OF HEMATOCRITS

The tests with seven species of fish to evaluate the relative reproducibility of the two hematocrit methods indicated that the centrifuge technique was somewhat superior (table 1). The standard deviations, for example, were smaller except in the case of the

hematocrits. Statistical tests showed that the differences between the means of the two methods were significant at the 0.01 level.

The electronic hematocrit can be corrected to compare with centrifuge value by using the regressions in figure 1 or correcting with mean differences between the methods (table 2). The second readings in the table will be discussed later.

TABLE 1.--Comparative reproducibility of electronic and centrifuge hematocrits

Species of fish	Hematocrit								Centrifuge minus electronic
	Electronic				Centrifuge				
	Number of hematocrits	Mean	Range	Standard deviation	Number of hematocrits	Mean	Range	Standard deviation	
Shovelnose sturgeon..	12	15.5	14.0-17.0	0.8	12	22.0	20.5-23.0	1.0	6.5
Spotted sucker.....	12	20.0	17.0-23.0	1.4	10	33.5	32.0-34.0	0.7	13.5
Northern redbhorse....	20	31.0	28.5-32.5	1.2	24	33.0	31.0-34.5	0.7	2.0
Brown bullhead.....	12	16.0	14.0-18.0	0.9	12	22.0	21.0-22.5	0.5	6.0
White bass.....	12	14.0	12.0-18.0	1.5	11	31.0	30.0-32.5	0.7	17.0
Largemouth bass.....	8	14.5	12.0-16.0	1.4	12	26.0	24.0-27.0	1.0	11.5
Walleye.....	9	28.0	25.0-29.0	1.4	12	40.0	39.0-41.5	0.7	12.0

shovelnose sturgeon. In contrast, the electronic hematocrits were consistently low and standard deviations were greater. Statistical tests for each species of fish demonstrated a significant difference between the results of the two hematocrit methods at the 0.01 level.

COMPARABILITY OF HEMATOCRITS

The electronic hematocrit was considered sufficiently reproducible. The differences between the means of the two methods suggested a need to correct electronic readings for individual and species variation. The regressions of electronic hematocrit on centrifuge hematocrit were determined for five species (fig. 1). The mean centrifuge values for the various species were as follows: rainbow trout, 40.4; brown trout, 33.0; brook trout, 37.1; lake trout, 35.7; and goldfish, 42.8. The mean electronic hematocrits, depending on species, were generally 20 to 60 percent lower than the means of the centrifuge

The regression technique is preferable because differences between hematocrit methods vary with the magnitude of the hematocrit. For example, an electronic hematocrit of 10 for rainbow trout corresponds to a centrifuge value of 25 and a reading of 30 to 49.5 when corrected by the regression. Using the mean difference as a correction, these values would be 27.5 and 47.5.

The regressions and the differences between means for rainbow and brown trout are similar (fig. 1, table 2). The 95-percent confidence intervals indicate that the same factor could be used to correct the electronic hematocrits of either salmonid. Separate corrections are required for other species.

The standard deviations of the differences between electronic and centrifuge hematocrits indicate a considerable variation about their means (table 2). Since the electronic hematocrit is relatively reproducible, and the measurements were made on the same

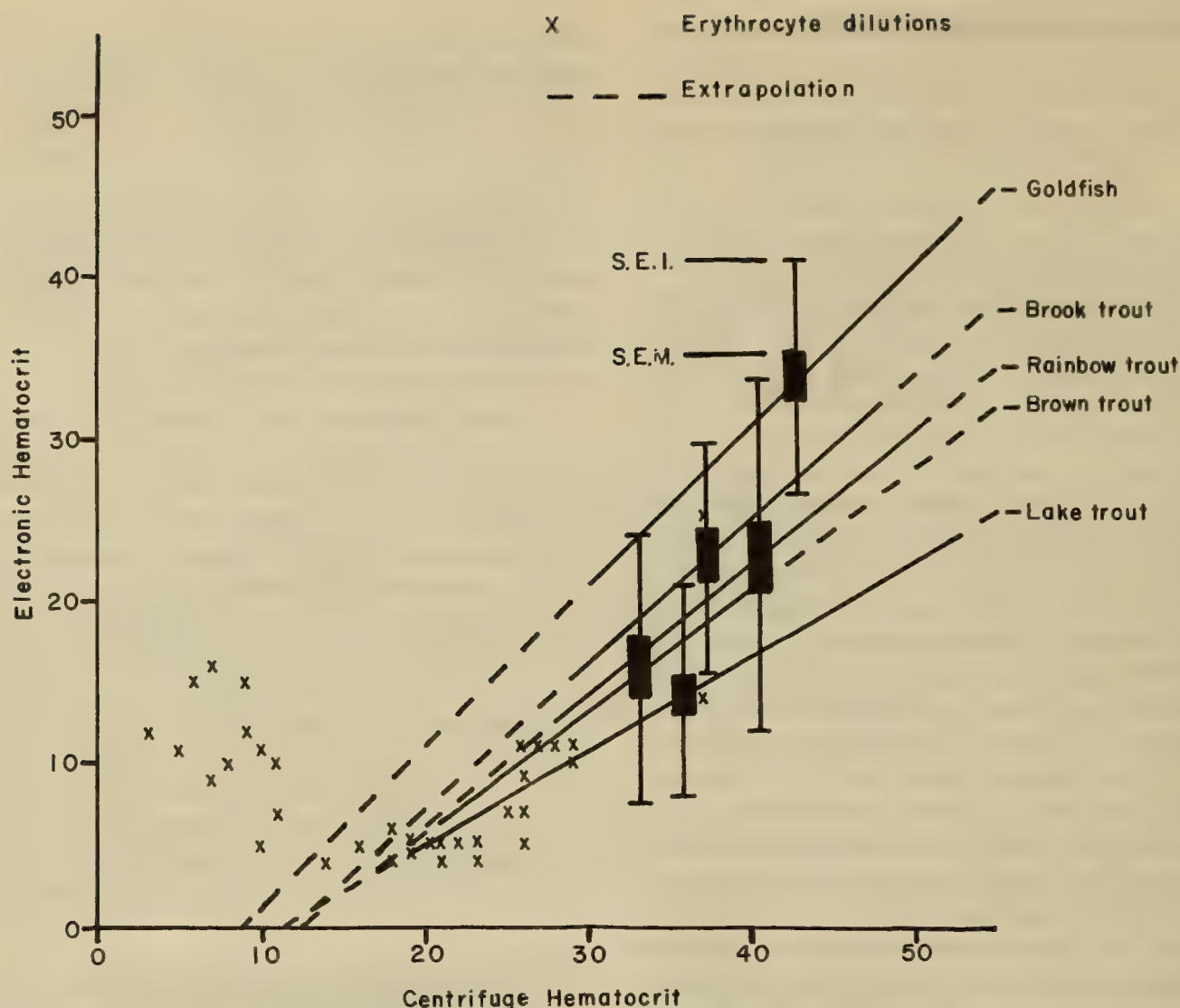


Figure 1.--Plot of electronic hematocrit against centrifuge hematocrit. Straight-line regression equations were computed for the data. S.E.I. and S.E.M. correspond to standard errors of individual and mean estimates respectively.

TABLE 2.--Mean differences between hematocrit methods for five species of fish

Species and electronic reading	Number of measurements	Mean difference; centrifuge minus electronic	Range of differences	Standard deviation	Standard error	95-percent confidence interval
Rainbow trout:						
First reading.....	27	17.4	9-26	5.0	0.97	15.4-19.4
Second reading.....	17	10.0	4-17	3.4	0.82	8.3-11.7
Brown trout:						
First reading.....	22	17.3	9-24	4.0	0.84	15.6-19.1
Second reading.....	22	8.3	2-15	3.7	0.80	6.6- 9.7
Brook trout:						
First reading.....	23	14.3	5-19	3.3	0.70	12.9-15.9
Second reading.....	23	6.3	1-12	3.1	0.65	5.0- 7.7
Lake trout:						
First reading.....	55	21.4	12-31	4.6	0.61	20.2-22.6
Second reading.....	55	10.9	3-17	3.2	0.43	10.0-11.8
Goldfish:						
First reading.....	30	9.1	1-16	3.4	0.62	7.8-10.4
Second reading.....	19	1.4	¹ 4- 5	2.6	0.60	0.1- 2.6

¹ All centrifuge hematocrits exceeded electronic readings except for three values in this group. The range extended from four units above to five units below the centrifuge hematocrit.

samples, the standard deviations suggest variations in sample conductivity due to factors other than percentage of cells. For this reason, the corrected electronic hematocrit should only be considered an estimate of the centrifuge hematocrit.

The extrapolations of regressions in figure 1 converge toward a common intercept as the hematocrit decreases. Unfortunately, anemic fish were unavailable to test the extrapolations, but plasma dilutions of rainbow erythrocytes were used to simulate anemia. The measurements of simulated samples were comparable to the predicted values at centrifuge hematocrits above 25; between 10 and 25, the readings ranged from 4 to 6 and increased with hematocrits less than 10. Thus the correction of electronic hematocrits of anemic fish may give misleading estimates of the centrifuge hematocrit. Readings of 4 to 6, however, might be used as gross, but rapid, indicators of anemia. Though severe anemia may give higher electronic values, the condition is usually detected by visual examination of the blood.

The conductivities of solutions containing 0.45- to 1.20-percent sodium chloride were tested on the YSI Electronic Hematocrit. A plot of the readings against the salt concentrations formed a curve similar to that shown in figure 1 for plasma dilutions of red blood cells. Salines of 0.60 to 0.75 percent sodium chloride gave electronic readings of 5 to 6. The similarity of the two curves suggests that the number of erythrocytes in blood affect the electronic hematocrit like changes in the electrolyte concentration. The electronic hematocrit of the plasma used to dilute the erythrocytes corresponded to that for a 0.86-percent solution of salt. A 0.86-percent saline has a freezing point similar to that for the bloods of most freshwater teleosts (Black, 1957). The data indicate that electronic measurements of plasma, or serum, might be used to estimate the electrolyte levels in fish blood.

Changes in the conductivity of a blood sample were observed upon re-reading. The value rose shortly after the first reading, increased to a peak, and then declined. The rate of change varied with individuals and

groups of fish. The apex was usually attained in 3 to 7 minutes and occasionally approximated the value obtained by the centrifuge method. An examination of blood within the glass cell indicated that the change in conductivity may be related to sedimentation and an unequal distribution of erythrocytes between the electrodes. This factor may be significant when measurements are delayed or when several samples are collected before reading.

Limited studies were conducted on the possibility of using the second electronic reading to estimate hematocrit. The mean differences between hematocrit methods, using the second reading, are shown in table 2. A correction factor for the second reading, like the first, is needed to estimate hematocrit. The factors are considerably smaller and have lower standard deviations, and standard errors. The second reading is time consuming and requires the use of heparinized blood. We found that blood could be drawn directly into the glass cell, without heparinization, and the first reading made within approximately 30 seconds. The blood began to clot shortly afterward, and a second reading was not usually possible. The alternative for using the second electronic reading is the preliminary collection of blood in heparinized capillary tubes; however, the technique detracts from the ease and speed of the electronic hematocrit.

Although the electronic determination of hematocrit is relatively rapid, time is lost in cleaning the glass cells. Several rinses in distilled water and drying with acetone were usually adequate. If blood dried or clotted in the cell, it was difficult to remove. If it remained, it changed sample volumes.

EFFECTS OF SALINE AND PROTEIN ON ELECTRONIC HEMATOCRIT

Experiments were conducted with rainbow trout and carp to determine whether variations in the electrolyte and protein concentration of fish blood influence electronic hematocrit (table 3 and fig. 2). The electronic hematocrit was inversely proportional to the concentration

TABLE 3.--Effect of various protein and sodium chloride concentrations on the deviation of electronic from centrifuge hematocrits

Species and concentration of sodium chloride	Protein concentrations of (grams per 100 cc.)--					
	1	2	3	5	7	9
Rainbow trout:						
0.65 percent.....	-1.5	--	-0.5	+1.0	--	+2.0
Do.....	-1.0	--	0.0	+0.5	--	+1.5
0.75 percent.....	--	--	-6.0	-2.5	--	-3.0
Do.....	--	--	-7.0	-6.0	--	-4.0
0.85 percent.....	-11.5	-12.0	-12.5	-9.5	-7.5	-5.0
Do.....	-14.5	-14.0	-11.0	-9.0	-7.5	-8.0
1.00 percent.....	-27.5	-24.5	-24.5	-22.0	-20.5	-18.0
Do.....	-26.0	-24.5	-23.5	-21.5	-19.5	-19.0
Carp:						
0.65 percent.....	0.0	--	+1.0	+2.5	--	+4.0
Do.....	-1.0	--	+1.5	+0.5	--	+4.5
0.85 percent.....	-14.0	-12.5	-11.5	-11.0	-7.5	-5.0
Do.....	-15.5	-11.5	-12.5	-9.0	-7.5	-8.0

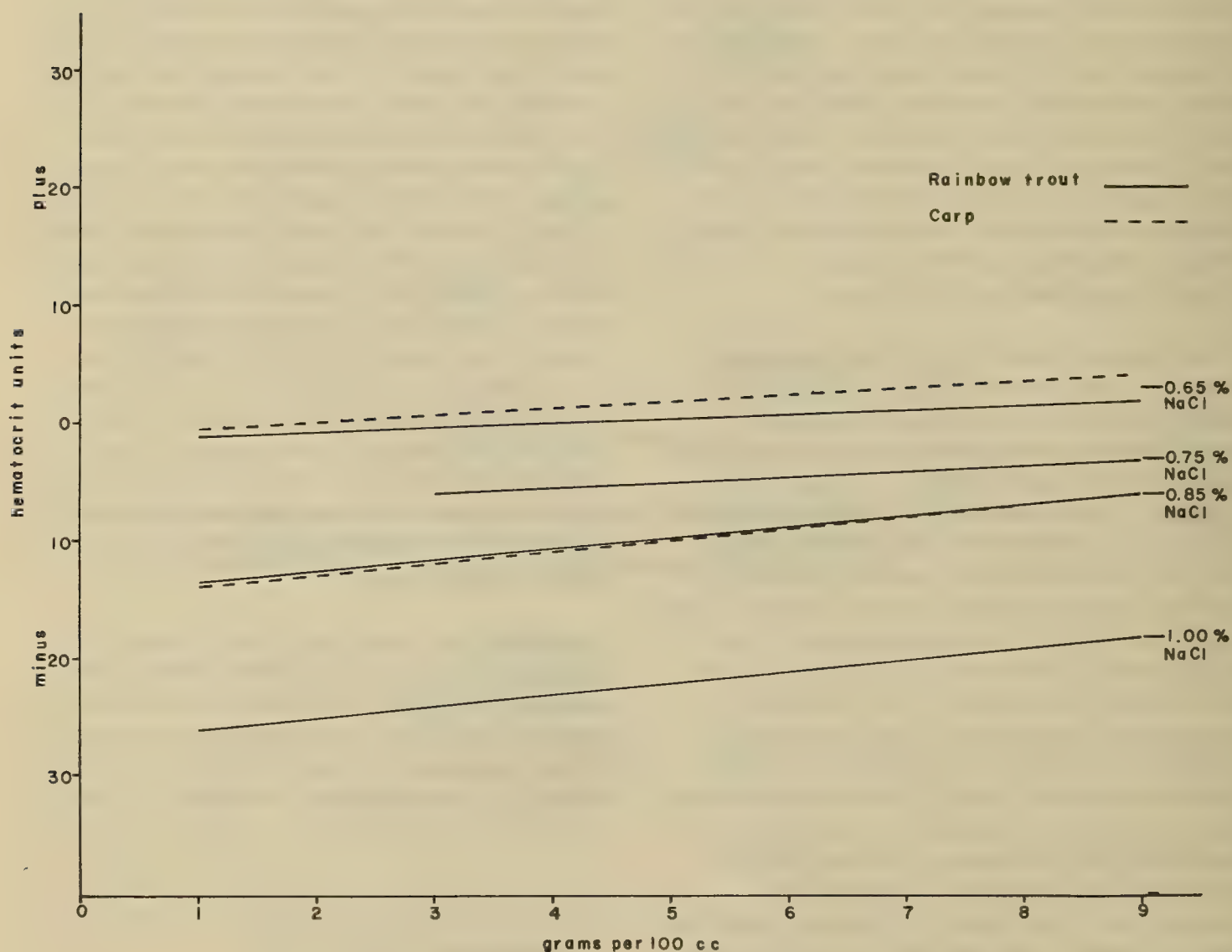


Figure 2.--Plot of hematocrit deviation against protein concentration for various levels of sodium chloride (as listed in table 3). Straight-line regression equations were computed for the data.

of sodium chloride and directly proportional to the level of protein. Suspensions of erythrocytes in salines containing 0.65- to 1.00-percent sodium chloride gave electrical

readings from 4.5 units above to 27.5 units below their corresponding centrifuge values. The concentration of protein had less effect on electronic hematocrits as the level of

sodium chloride declined. The readings increased with protein concentration by 7.0 to 9.5 units at 1-percent and by 2.5 to 5.5 units at 0.65-percent sodium chloride. The difference in conductivity between erythrocytes of rainbow trout and carp were relatively small.

The deviation of electronic readings due to variations in the electrolyte content of fish blood can be estimated from Black's (1957) review of freezing points of bloods for several teleosts. The values are related to species and osmotic concentration of the external environment. The bloods of most fish in fresh-water froze between -0.49 and -0.67°C . According to Hodgman, Weast, and Selby (1960), salines containing approximately 0.80- to 1.15-percent sodium chloride have similar freezing points. Phillips et al. (1956) suggested that nutritional conditions may influence the range of variability in the concentration of inorganic elements in brown trout blood. Seasonal changes in these constituents were observed in the blood of smallmouth bass by Shell (1961). Considering these data it is possible that the electronic hematocrit readings in tables 1 and 2 were affected by blood electrolytes.

The quantities of serum proteins of fish vary according to sex, reproductive condition, food, disease, oxygen depletion, and other factors (Booke, 1964). Some concentrations of protein reported per 100 cc. of blood include 1.8 to 4.5 grams for smallmouth bass (Shell, 1961), 2.01 to 2.04 grams for brown and brook trout (Phillips et al., 1957), 3.33 to 4.70 grams for rainbow trout (Meisner and Hickman, 1962), and 3.18 to 7.96 grams for brook trout (Brooke, 1964).

Assuming a physiologic level of electrolytes in the blood, electronic hematocrits could deviate 4 or 5 units depending on protein concentration. This may not be a comparatively large error, but the degree to which bovine albumin simulates blood proteins of fish with respect to conductivity is not known.

Though the regressions in figure 2 demonstrate the effect of electrolyte and protein concentration on conductivity, they do not correspond to results obtained by Kern, Wurzel, and Okada (1961) on human blood.

They found that electronic values corresponded to centrifuge hematocrits at a protein concentration of 7.4 grams per 100 cc. (bovine albumin dissolved in physiological saline). Their regression extended from -9.9 hematocrit units at 2.5 grams of protein per 100 cc. to $+6.6$ units at 11.0 grams per 100 cc. The inconsistency between data on humans and on fish may be related to the preparation of cellular suspensions or, perhaps, to an additional variable such as the relative size of fish erythrocytes. The red blood cells of fish are generally larger than and shaped differently from those of mammals, and it is conceivable that the comparatively low electronic hematocrits are partially correlated with differences in their insulatory characteristics (Smith, 1952; Hawk, Oser, and Summer-son, 1954; and Mott, 1957). Larger cells may have less surface area per volume and have an effect on the electronic hematocrit which is analogous to that of fewer cells.

CONCLUSIONS

The YSI Electronic Hematocrit gives relatively rapid and reproducible results with fish blood. Centrifuge hematocrits greater than 25 can be estimated by correcting the electronic readings. Electronic readings were not correlated linearly with centrifuge values which were less than 25. They may be used, however, as gross but rapid indicators of anemia. Observations on the conductivities of plasma and various-strength salines suggest the instrument could be used to measure electrolyte levels in fish blood. The conductivities of blood samples changed with time and became closer to centrifuge values than the initial readings. The estimations of hematocrits from the later readings were time consuming and required heparinized blood. The electrical measurement may be influenced by variations in blood electrolytes and proteins, and by the average size of erythrocytes.

SUMMARY

Experiments with a YSI, Model 30 Electronic Hematocrit indicate that measurements of the conductivity of fish blood can be used to estimate hematocrit. The method is relatively

rapid and reproducible, but readings must be corrected to estimate centrifuge hematocrits of approximately 25 or more. The individual differences between the two methods varied widely and the differences between means of the methods deviated according to species. The electronic hematocrits do not change proportionately with centrifuge values less than 25, and are not easily corrected. The disproportionate change of electronic readings in the low hematocrit range may prove useful as a gross but rapid method for detecting anemia. Variations in serum electrolytes and proteins of fish, and in the average size of erythrocytes may interfere seriously with electrical measurements of hematocrits.

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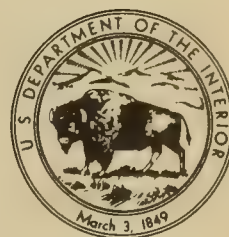
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(Reports 1 and 2 are in one cover.)

1. Laboratories and Methods for Screening Fish-Control Chemicals, by Robert E. Lennon and Charles R. Walker. (Bureau Circular 185.) 1964. 15 p.
2. Preliminary Observations on the Toxicity of Antimycin A to Fish and Other Aquatic Animals, by Charles R. Walker, Robert E. Lennon, and Bernard L. Berger. (Bureau Circular 186.) 1964. 18 p.

(Reports 3 through 8 are in one cover.)

3. Minimum Lethal Levels of Toxaphene as a Piscicide in North Dakota Lakes, by Dale L. Henegar. (Resource Publication 7.) 1966. 16 p.
4. Effects of Toxaphene on Plankton and Aquatic Invertebrates in North Dakota Lakes, by Robert G. Needham. (Resource Publication 8.) 1966. 16p.
5. Growth Rates of Yellow Perch in Two North Dakota Lakes After Population Reduction with Toxaphene, by Donald C. Warnick. (Resource Publication 9.) 1966. 9 p.
6. Mortality of Some Species of Fish to Toxaphene at Three Temperatures, by Mahmoud Ahmed Mahdi. (Resource Publication 10.) 1966. 10 p.
7. Treatment of East Bay, Alger County, Michigan, with Toxaphene for Control of Sea Lampreys, by William E. Gaylord and Bernard R. Smith. (Resource Publication 11.) 1966. 7p.
8. Effects of Toxaphene on Fishes and Bottom Fauna of Big Kitoi Creek, Afognak Island, Alaska, by William R. Meehan and William L. Sheridan. (Resource Publication 12.) 1966. 9 p.

(Reports 9 through 11 are in one cover.)

9. Relation of Chemical Structure to Fish Toxicity in Nitrosalicylanilides and Related Compounds, by Charles R. Walker, Roland J. Starkey, and Leif L. Marking. (Resource Publication 13.) 1966. 12p.
10. Evaluation of p,p' -DDT as a Reference Toxicant in Bioassays, by Leif L. Marking. (Resource Publication 14.) 1966. 10p.
11. Evaluation of an Electronic Method of Measuring Hematocrits of Fish, by Richard A. Schoettger and Arnold M. Julin. (Resource Publication 15.) 1966. 11p.

(Reports 12 through 17 are in one cover.)

12. Toxicity of MS-222 to Selected Fishes, by Leif L. Marking. (Resource Publication 18.) 1967. 10p.
13. Efficacy of MS-222 as an Anesthetic on Four Salmonids, by Richard A. Schoettger and Arnold M. Julin. (Resource Publication 19.) 1967. 15p.
14. Method for Determining MS-222 Residues in Fish, by Charles R. Walker and Richard A. Schoettger. (Resource Publication 20.) 1967. 10p.
15. Residues of MS-222 in Four Salmonids Following Anesthesia, by Charles R. Walker and Richard A. Schoettger. (Resource Publication 21.) 1967. 11p.
16. Annotated Bibliography on MS-222, by Richard A. Schoettger. (Resource Publication 22.) 1967. 15p.
17. MS-222 as an Anesthetic for Channel Catfish: Its Toxicity, Efficacy, and Muscle Residues, by Richard A. Schoettger, Charles R. Walker, Leif L. Marking, and Arnold M. Julin. (Resource Publication 33.) 1967. 14p.

Fish Control Laboratories
Bureau of Sport Fisheries and Wildlife
U.S. Department of the Interior
P.O. Box 862
La Crosse, Wisconsin 54602

INVESTIGATIONS IN FISH CONTROL

12. Toxicity of MS-222 to Selected Fishes
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United States Department of the Interior, Stewart L. Udall, *Secretary*
Stanley A. Cain, *Assistant Secretary for Fish and Wildlife and Parks*
Fish and Wildlife Service, Clarence F. Pautzke, *Commissioner*
Bureau of Sport Fisheries and Wildlife, John S. Gottschalk, *Director*
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FOREWORD

The Food and Drug Administration of the U.S. Department of Health, Education, and Welfare advised the Bureau of Sport Fisheries and Wildlife that certain drugs and chemicals used in the culture and management of game fishes must be cleared for continued use. We must demonstrate to the FDA that fish exposed to the compounds pose no danger, because of residues, to people who might eat them.

MS-222 is among the drugs mentioned because it is commonly used to anesthetize fish during marking or tagging, spawning by stripping, and transportation. The information necessary for clearance includes the toxicity of the drug to fish, its efficacy as an anesthetic, and its residues in fish. FDA also requires that fish of various sizes be represented in tests of the drug at three or more temperatures in waters of different qualities. Accordingly, we investigated the toxicity of MS-222 to eight species of game fish, its efficacy on four trouts, and its residues in the same trouts (emphasis was placed on residues in muscle because muscle is the principal tissue eaten by people).

MS-222 was synthesized by Maurice Sandoz (Sandoz, Ltd., Basle, Switzerland, and Hanover, N.J.) during his search about 45 years ago for a synthetic substitute for cocaine. It has been used as a local anesthetic in preparations for humans, but its general anesthetic activity against cold-blooded animals was recognized early. Within the past decade, its use as an anesthetic in fish culture and fish management has become widespread in the United States and abroad. For example, the millions of lake trout stocked in the upper Great Lakes in the past several years were first anesthetized with MS-222 and fin-clipped.

The considerable variety of common and chemical names applied to MS-222 in the literature causes some confusion. The names include:

MS-222.	Metacaine methanesulphonate.
MS-222 Sandoz.	<u>m</u> -amino ethyl benzoate.
M.S.-222 Sandoz.	Ethyl <u>m</u> -aminobenzoate.
TS-222.	Methane sulfonic acid salt of
TS-222 Sandoz.	<u>meta</u> -amino ethyl benzoate.
Tricaine.	Methanesulphonate of
Tricaine - Sandoz.	<u>meta</u> -aminobenzoic acid.
Tricaine methanesulfonate.	Methanesulphonate <u>meta</u> -amino-
Metacaine.	benzoic acid ethyl ester.

Of these names, we used MS-222, tricaine methanesulfonate, and methanesulphonate of meta-aminobenzoic acid ethyl ester.

Robert E. Lennon, Director
Fish Control Laboratories

12. Toxicity of MS-222 to Selected Fishes

By Leif L. Marking



United States Department of the Interior, Stewart L. Udall, *Secretary*
Stanley A. Cain, *Assistant Secretary for Fish and Wildlife and Parks*
Fish and Wildlife Service, Clarence F. Pautzke, *Commissioner*
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TOXICITY OF MS-222 TO SELECTED FISHES

By Leif L. Marking, Chemist,
Bureau of Sport Fisheries and Wildlife
La Crosse, Wis.

Abstract.--Toxicity of MS-222 to rainbow trout, brown trout, brook trout, lake trout, northern pike, bluegill, largemouth bass, and walleye of various sizes was determined in 15-, 30-, and 60-minute and 24-, 48-, and 96-hour static bioassays at selected temperatures. Twenty-four-hour LC₅₀ values for the eight species ranged from 33.8 to 63.0 p.p.m. Exposures longer than 24 hours had little effect on toxicity. Small fish of a species were more sensitive to the drug than large ones, and trout were more sensitive at warmer temperatures. Safety indexes were calculated on the basis of the brief exposures.

Bové (1962) identified MS-222 as the methanesulphonate of meta-aminobenzoic acid ethyl ester. The compound is a fine, white crystalline powder with a molecular weight of 261.3 and a melting point of 145° to 150° C. It leaves only minimum traces of ash upon ignition and is free from chlorides, sulfates, alkaloïds, and heavy metals. Less than 0.5 percent of its weight is lost upon heating to 103° C. It is soluble to 11 percent in water and forms a clear, colorless, acid, and relatively stable solution. He also reported that a 10-percent solution retained its potency during 3 days' storage, but decreased 5 percent in activity after 10 days. Exposure of a solution to light caused a change in color to yellow or brown, but its activity was not affected.

The molecular structure of MS-222, although not defined in literature, possibly conforms to figure 1. Since it is highly soluble (up to 22.5 percent in our laboratory) a proton bond probably exists between the amine group on the benzoate structure and the hydrogen on the methane sulfonate. In the ionization of the compound, the hydrogen probably splits off the sulfonate group, being attracted to the nitrogen of the amine group.

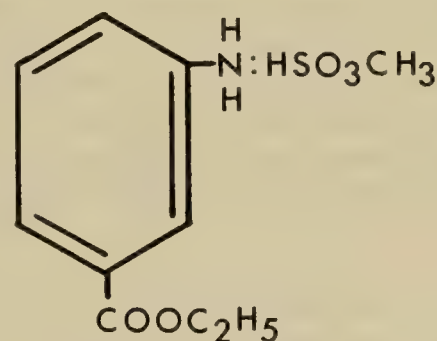


Figure 1.--Structure of MS-222.

MS-222 is an effective anesthetic for fish and other coldblooded animals (McFarland, 1959; Eisler and Backiel, 1960; Bové, 1962; Schoettger and Julin, 1966). Rothlin (1932) stated that the drug is three times less toxic to coldblooded animals than novocaine and ten times less toxic than cocaine.

There are reports, however, that under certain circumstances MS-222 is toxic to certain species or strains of fish. Marvin Smith (Regional Fishery Management Biologist at Atlanta, in a memorandum report to the Branch of Game Fish and Hatcheries, June 12, 1956) recommended that MS-222 be bioassayed for toxicity to rainbow trout under

various water qualities. Thompson (1959) observed a 95-percent mortality among cut-throat trout exposed to 50 p.p.m. of MS-222 for 5 hours. Others have noted that excessive exposures cause mortalities among fish (Nelson, 1953; Parkhurst and Smith, 1957; and Eisler and Backiel, 1960).

The purpose of this investigation was to define the concentrations of MS-222 which are toxic to eight species of freshwater, game fish of various sizes. The experimental conditions included different water qualities and durations of exposure at four temperatures. Safety indices for rainbow trout were established.

METHODS AND MATERIALS

Eight species of fish were obtained from various fish hatcheries (table 1). The three size groups included small fingerlings 1 to 3 inches long, an intermediate group of 3 to 5 inches, and a group 6 to 9 inches long. All fish were quarantined for 10 days and if judged acceptable, were then acclimated to the temperatures and conditions of the tests of MS-222.

The static bioassays of the anesthetic against 2-inch fish were conducted in 5-gallon glass jars (Lennon and Walker, 1964). At least 120 fish of this size were included in each test of 10 concentrations of drug. Ten fish were exposed to each concentration, and 20 served as controls. The tests against

4- and 9-inch fish were made in aerated, polyethylene tanks which contained 45 liters of anesthetic solution. Ten of the larger fish were exposed to each of 5 concentrations per test, and 10 served as controls.

Water temperatures were controlled by placing the bioassay vessels in water baths at 7^o, 12^o, 17^o, and 22^o C. Water hardnesses of 10, 42, and 176 p.p.m. were arranged by adding different amounts of the mixture of reconstituting salts to deionized water (table 2).

TABLE 2.--Water qualities obtained with various amounts of reconstituting salts in deionized water

Water quality	Salts in mg./l.				Total hardness (p.p.m.)	Total alkalinity (p.p.m.)	pH
	NaHCO ₃	CaSO ₄	MgSO ₄	KCl			
Soft.....	12	7.5	7.5	.75	10	10	6.72
Standard..	48	30	30	3	42	30	6.75
Hard.....	192	120	120	12	176	116	7.70

Concentrated stock solutions of MS-222 were prepared daily in deionized water. Aliquots of the stock were added to bioassay vessels following the introduction of fish.

The responses of fish to MS-222 were recorded for several hours after exposure began and daily thereafter throughout the bioassay. It was possible during the tests to differentiate between dead and anesthetized fish. Dead specimens were removed immediately. Fish which remained in anesthesia throughout a test were placed in fresh water until they recovered. The times for recovery and survival were noted.

The data on survival and death of fish exposed to selected amounts of MS-222 were analyzed according to the method of Litchfield and Wilcoxon (1949) to define concentrations which produce 50-percent mortality (LC₅₀). In addition, the variance and the 95-percent confidence intervals were determined.

RESULTS

MS-222 demonstrated a definite and consistent toxicity to the eight species of fish. Furthermore, the concentrations permitting survival or causing mortality can be predicted with a high degree of confidence.

TABLE 1.--Fishes used in toxicity tests of MS-222

	Source
Rainbow trout (<i>Salmo gairdneri</i>)....	Manchester National Fish Hatchery, Iowa.
Brown trout (<i>Salmo trutta</i>).....	Manchester National Fish Hatchery, Iowa.
Brook trout (<i>Salvelinus fontinalis</i>)..	State Fish Hatchery at Osceola, Wis.
Lake trout (<i>Salvelinus namaycush</i>)..	Jordan River National Fish Hatchery, Mich., and St. Croix Falls, Wis.
Northern pike (<i>Esox lucius</i>).....	Garrison Dam National Fish Hatchery, N. Dakota and Yankton National Fish Hatchery, S. Dakota.
Bluegill (<i>Lepomis macrochirus</i>).....	Lake Mills National Fish Hatchery, Wis.
Largemouth bass (<i>Micropterus salmoides</i>).....	Genoa National Fish Hatchery, Wis.
Walleye (<i>Stizostedion vitreum</i>).....	Garrison Dam National Fish Hatchery, N. Dakota.

Species and sizes of fish

Among the four trouts, the toxicity of MS-222 at a given temperature is dependent on variables such as species, size, and duration of exposure. Lake trout are the most sensitive and brook trout the least (table 3). Brown and rainbow trout are equal in their sensitivity and intermediate to lake and brook trout.

Larger fish of a species are usually more resistant to MS-222 than smaller fish. This is apparent with rainbow trout as the 24-hour LC_{50} increases from 39 p.p.m. for 2-inch fish to 52 p.p.m. for 9-inch fish. The trend is similar in 48- and 96-hour assays. The larger lake trout and brown trout also showed greater resistance than smaller fish at all periods of observation.

There was little difference in toxicity among brook trout of different sizes, but 6-inch

specimens appeared to be more sensitive than 4-inch fish. It turned out, however, that the larger fish had become infected with furunculosis just prior to the bioassay, and quite possibly the disease lowered their resistance. A repeat trial with healthy brook trout was not possible.

Exposures beyond 24 hours do not have a pronounced effect on toxicity. In several instances the toxicity at 96 hours was identical to the 24-hour LC_{50} . Among the trouts, only 2-inch brown trout exhibited a significant difference, with a 20-percent drop in resistance between 24 and 96 hours.

Other species in the toxicity trials included northern pike, bluegill, largemouth bass, and walleye (table 4). Two-inch bluegill, largemouth bass, and walleye were only slightly less resistant than small brook trout. The larger specimens of bluegill and largemouth

TABLE 3.--Toxicity of MS-222 to salmonids at 12° C.

Species and lot	Average weight (grams)	Approximate length (inches)	At 24 hours		At 48 hours		At 96 hours	
			LC_{50}	95-percent confidence interval	LC_{50}	95-percent confidence interval	LC_{50}	95-percent confidence interval
Rainbow trout:								
Lot 27.....	1.9	2	39.0	37.8-40.2	39.0	37.8-40.2	38.4	37.0-39.7
Lot 102.....	23.0	6	47.0	44.3-49.8	47.0	44.3-49.8	47.0	44.3-49.8
Lot 171.....	146.0	9	52.0	49.7-54.3	52.0	49.7-54.3	50.5	48.6-52.5
Brown trout:								
Lot 26.....	2.6	2	38.5	37.0-40.0	37.5	30.5-46.1	31.0	27.7-34.2
Lot 64.....	14.3	4	44.0	42.3-45.8	43.8	42.1-45.6	43.8	42.1-45.6
Lot 64.....	27.0	6	45.6	44.3-47.0	44.8	42.7-47.0	43.0	40.2-46.0
Brook trout:								
Lot 79.....	12.5	3	50.7	48.3-53.2	50.0	46.7-53.5	50.0	46.7-53.5
Lot 80.....	20.0	4	52.2	49.7-54.8	52.2	49.7-54.8	49.1	47.2-51.1
Lot 81.....	37.5	6	51.2	50.2-52.2	51.2	50.2-52.2	50.0	49.0-51.0
Lake trout:								
Lot 78.....	2.0	2	33.8	33.1-34.5	33.0	31.7-34.3	32.0	30.2-33.9
Lot 133.....	5.6	3	35.8	34.8-36.9	35.8	34.8-36.9	35.8	34.8-36.9
Lot 162.....	35.0	7	39.8	39.0-40.6	39.2	38.0-40.4	38.5	37.4-39.7

TABLE 4.--Toxicity of MS-222 to warm-water fish at 12° C.

Species and lot	Average weight (grams)	Approximate length (inches)	At 24 hours		At 48 hours		At 96 hours	
			LC_{50}	95-percent confidence interval	LC_{50}	95-percent confidence interval	LC_{50}	95-percent confidence interval
Northern pike:								
Lot 35B.....	0.5	1	56.0	52.8-59.4	52.0	49.5-54.6	48.0	44.0-52.3
Lot 41.....	1.8	2						
Bluegill:								
Lot 112.....	1.3	2	45.7	44.8-46.6	45.7	44.8-46.6	45.7	44.8-46.6
Lot 120.....	2.8	3	46.9	46.0-47.8	46.9	46.0-47.8	46.9	46.0-47.8
Largemouth bass:								
Lot 57.....	0.5	1	42.0	40.8-43.3	42.0	40.8-43.3	39.4	36.5-42.6
Lot 70.....	5.2	4	47.0	44.3-49.8	46.5	44.7-48.4	46.5	44.7-48.4
Lot 91.....	63.0	7	61.5	55.9-67.6	57.9	55.1-60.8	57.9	55.1-60.8
Walleye:								
Lot 55.....	0.7	2	49.0	46.2-51.9	48.5	45.8-51.4	48.5	45.8-51.4

bass were more resistant than smaller ones. For example, the LC_{50} for 7-inch largemouth bass was 50 percent greater than for 1-inch bass. Also, all sizes of bluegill, largemouth bass, and walleye were affected very little by exposures longer than 24 hours.

The northern pike were of two sizes, but the data were insufficient for separate treatment and they were combined to facilitate statistical evaluation. Both sizes were healthy, but it is difficult to bioassay pike because they are voracious and turn to cannibalism unless other live food is available. Their possible hunger stress was demonstrated by decreased resistance at 96 hours.

Effects of temperature

The effects of temperature on the toxicity of MS-222 to rainbow trout which averaged 0.6 g. and 1.6 in. were evaluated (table 5). The trout were slightly more resistant at 7° than at 12° and 17° C. There was little difference between the LC_{50} values at 24 and 96 hours at a given temperature.

TABLE 5.--Toxicity of MS-222 to rainbow trout at selected temperatures

Temperature	At 24 hours		At 48 hours		At 96 hours	
	LC_{50}	95-percent confidence interval	LC_{50}	95-percent confidence interval	LC_{50}	95-percent confidence interval
7°C.....	40.0	36.6-43.7	39.5	37.6-41.6	39.5	37.6-41.6
12°C.....	37.2	36.3-38.1	37.2	36.3-38.1	37.2	36.3-38.1
17°C.....	37.5	35.6-39.6	37.2	36.7-37.7	36.5	35.9-37.1

MS-222 was more toxic to small bluegills at 12° or 22° C. than at 17° (table 6). The differences, however, between LC_{50} values obtained at 3 temperatures in 24-, 48-, and 96-hour tests were not large. The fish tested at 22° were slightly smaller than the others, which may account for their greater sensitivity.

TABLE 6.--Toxicity of MS-222 to bluegills at selected temperatures

Temperature	Lot No.	Average weight grams	Average length inches	At 24 hours		At 48 hours		At 96 hours	
				LC_{50}	95-percent confidence interval	LC_{50}	95-percent confidence interval	LC_{50}	95-percent confidence interval
12°C.....	131	1.1	1.7	43.0	41.7-44.3	43.0	41.7-44.3	43.0	41.7-44.3
17°C.....	131	1.1	1.7	45.0	44.1-45.9	44.9	43.6-46.2	44.0	42.7-45.3
22°C.....	152	0.8	1.6	39.8	39.0-40.6	39.8	39.0-40.6	39.8	39.0-40.6

Effects of water quality

The effects of water hardness on the toxicity of MS-222 were established using rainbow trout which averaged 1.1 grams and 1.9 inches (table 7). The fish were from the same lot, and they were exposed concurrently to the anesthetic at three hardnesses. Results were consistent and accurate at 42 and 176 p.p.m. total hardness, but variable at 10 p.p.m. which necessitated several repeats for reliability. The inconsistent mortalities at lower concentrations indicated an erratic action of the drug in soft water. Partial mortality occurred at 33 and 34 p.p.m. in 24 hours, but no mortality occurred at 36 p.p.m. A previous, 24-hour test indicated considerably higher mortalities at 30 and 33 p.p.m. than at 38 p.p.m.

Recovery

The recovery of fish after 96-hour exposures was observed. At relatively low concentrations of MS-222, all fish recovered to some extent during the bioassay and completely during the recovery period immediately following the 96-hour exposure. At intermediate concentrations, which resulted in a partial kill during a 96-hour exposure, some of the survivors recovered partially previous to terminating the bioassay. Very few of the surviving fish died during the recovery period following exposure. The numbers of fish and the rates at which they recovered from anesthesia and survived exposures to the intermediate concentrations are listed in table 8.

The trout species recovered faster from partial-kill concentrations of MS-222 than the other fishes. Recovery was considered complete when specimens regained equilibrium and the ability to swim against a current.

TABLE 7.--Toxicity of MS-222 to rainbow trout in selected water qualities at 12° C.

Total hardness	Total alkalinity (p.p.m.)	pH	At 24 hours		At 48 hours		At 96 hours	
			LC ₅₀	95-percent confidence interval	LC ₅₀	95-percent confidence interval	LC ₅₀	95-percent confidence interval
10 p.p.m.....	10	6.7	39.0	37.6-40.3	38.8	37.5-40.2	38.0	36.9-39.2
42 p.p.m.....	30	6.8	40.0	39.1-40.8	39.0	38.1-40.0	39.0	38.1-40.0
176 p.p.m.....	116	7.7	39.0	38.1-40.0	38.0	36.4-39.8	37.9	36.5-39.2

TABLE 8.--Recovery from anesthesia among fish exposed to concentrations of MS-222 causing partial kills within 96 hours at 12° C.

Species and length	Partial kill concentration (p.p.m.)	Number of fish		Minutes to recover in fresh water
		Surviving at 96 hours	Recovering after 96 hours	
Rainbow trout:				
2 inches.....	34-44	37	34	2- 5
6 inches.....	46-48	10	10	1- 2
9 inches.....	48-50	15	15	3- 4
Brown trout:				
2 inches.....	20-40	25	25	2- 8
4 inches.....	40-46	6	6	2- 4
6 inches.....	44-46	6	6	3- 13
Brook trout:				
3 inches.....	44-50	31	31	2- 9
4 inches.....	46-52	29	29	2- 20
6 inches.....	46-54	25	25	4- 15
Lake trout:				
2 inches.....	32-36	9	8	1- 2
3 inches.....	33-38	29	28	4- 12
7 inches.....	36-40	24	24	10- 30
Northern Pike:				
Bluegill:				
2 inches.....	47-54	14	13	2- 30
2 inches.....	44-46	18	18	10- 30
3 inches.....	45-47	29	29	2- 14
Largemouth bass:				
4 inches.....	44-56	10	10	5- 60
7 inches.....	46-56	45	45	5-120
Walleye:				
2 inches.....	40-50	34	32	60-180

Rainbow trout exposed to 34 to 50 p.p.m. for 96 hours recovered within 1 to 5 minutes when removed to fresh water. In contrast, walleye exposed to 40 to 50 p.p.m. for 96 hours required 60 to 180 minutes to recover. Of the 356 fish removed to and recovering in fresh water, only 8 subsequently died. Among the survivors, the 2-inch rainbow trout exposed to 34 to 44 p.p.m. of the drug for 96 hours and removed to fresh water were observed for 7 days. There was no mortality; they fed readily and behaved similarly to controls.

Safety indexes

Rainbow trout averaging 1.4 grams and 2 inches were exposed to MS-222 in brief bioassays to determine safety indexes. A safety index refers to the margin between efficacy and mortality. It is expressed by the quotient of a lethal concentration and an effective concentration.

Safety indexes for rainbow trout in table 9 were derived from LC₅₀ and EC₅₀ values. The EC₅₀ here relates to the concentration of drug which produces total loss of equilibrium in half of the specimens (Schoettger and Julin, 1966). The best results were obtained in 15-, 30-, and 60-minute exposures. Shorter exposures may be desirable from the point of view of field practice, but they give less accurate results. The fish do not die uniformly or quickly enough. Longer exposures of 1 to 6 hours were not meaningful because some fish recovered from anesthesia; others died at concentrations close to the effective concentration.

The maximum safety index (M.S.I.) is the quotient of the LC₁ and the EC₉₉. The values were extrapolated from the regressions used in determining the LC₅₀ and EC₅₀. The M.S.I. is lower than the safety index and is biased in favor of greater safety.

TABLE 9.--Safety and maximum safety indexes of MS-222 against 2-inch rainbow trout in brief exposures at 12° C.

Exposure	Water hardness (p.p.m.)	Safety index			Maximum safety index		
		LC (p.p.m.)	EC ₅₀ (p.p.m.)	Index LC ₅₀ /EC ₅₀	LC ₁ (p.p.m.)	EC ₉₉ (p.p.m.)	Index LC ₁ /EC ₉₉
15 minutes.....	42	64.6	32.0	2.0	54.0	38.2	1.4
30 minutes.....	42	56.8	31.8	1.8	49.5	38.5	1.3
60 minutes.....	42	55.5	29.2	1.9	46.0	36.8	1.3
15 minutes.....	10	64.0	34.0	1.9	50.0	47.5	1.1
30 minutes.....	10	58.0	31.5	1.8	46.5	45.0	1.0
60 minutes.....	10	54.2	31.2	1.7	44.0	40.4	1.1

The S.I. and M.S.I. derived for MS-222 against small rainbow trout demonstrate that the shorter exposures are safer for the fish than longer exposures (table 9). This was true in waters with hardnesses of 10 and 42 p.p.m. The data also show that the drug is not as safe in soft water as in harder water.

DISCUSSION

Toxicity of MS-222 to the eight species of fish depended on the concentration of drug and the duration of exposure. There was a straight-line relation between concentration and time of exposure within the initial 24 hours. Beyond 24 and up to 96 hours a given concentration showed little or no difference in toxicity. For example, the 15- and 30-minute and 24- and 96-hour LC₅₀ values for 2-inch rainbow trout were 64.6, 55.5, 39.0, and 38.4 p.p.m. respectively. The 24- and 96-hour LC₅₀ values for some species were identical, such as 47 p.p.m. for 6-inch rainbow trout.

The reasons for identical LC₅₀ values at 24 and 96 hours may include a decrease of concentration by absorption and metabolism in the fish, some natural degradation of the drug in solution, and greater activity of the chemical on fish within the early hours of exposure.

It is noteworthy that the LC₅₀ values for the eight species did not differ greatly. The range of 24-hour LC₅₀ values for the more susceptible lake trout to the more tolerant largemouth bass was 33.8 to 63.0 p.p.m.

Among the salmonids, brook trout were the more resistant to MS-222. Interestingly, Walker and Schoettger (1966), detected lower residues of the drug in brook trout than in

rainbow, brown, and lake trout upon withdrawal from effective anesthesia. This may explain, in part, the higher resistance of the species to the anesthetic.

Water hardness did not influence the toxicity of MS-222 to rainbow trout, except that results at 10 p.p.m. were erratic. Parkhurst and Smith (1957) observed that rainbows react differently in various water supplies to the same concentrations of the drug. These inconsistencies suggest that the fish may not be exposed to MS-222 as safely in soft water as in hard water.

Sandoz Pharmaceuticals (in an undated leaflet "Toxicity of MS-222 to fish and frogs", 2 p.) stated that the therapeutic index (LC₁ / EC₉₉) of MS-222 for rainbow trout was 1.57. The LC₁, however, was defined in a 15-minute exposure of fish whereas the EC₉₉ was defined in 3- to 4-minute exposures. Since the exposures for LC and EC differ, their index is not comparable to our safety indexes.

The S.I. (LC₅₀ / EC₅₀) and the M.S.I. (LC₁ / EC₉₉) displayed similar trends throughout the bioassays of MS-222 with one significant exception. There was little difference between the S.I. for rainbow trout in hard and soft water, but there was a significant difference between the M.S.I. The index was higher in the hard water. The slope of the regressions for LC and EC in soft water was less than in hard water, and the LC₁ was therefore smaller and the EC₉₉ greater. These relations suggest that MS-222 is safer to rainbow trout in hard water than in soft. This was confirmed by Schoettger and Julin (1966) in their studies on the efficacy of the drug to rainbows in waters of various qualities. It is advisable to calculate both the S.I. and the

M.S.I. because one may suggest a possible hazard when the other doesn't.

The speed and degree of recovery of fish from anesthesia are important. Complete recovery from brief exposures is rapid, and it is proportional to the concentration of MS-222 and the duration of exposure according to Klontz (1964). This is not wholly the case in long exposures up to 96 hours. The concentrations of drug in long exposures did not hold all fish at a certain stage of narcosis for 96 hours. Some fish were recovering from anesthesia each day after the first 24 hours.

The trout recovered from anesthesia more rapidly than the other species. In fact, most fish recovered rapidly, and the extended recovery times for some species reflect the very slow recoveries of a few individuals.

Sublethal exposures to MS-222 did not seem to harm fish. Rainbow trout which survived partial-kill concentrations were observed for 7 days. They fed and behaved as well as controls.

CONCLUSIONS

MS-222 demonstrated a definite and consistent toxicity to eight species of fish in 24- to 96-hour bioassays. The range of 24-hour LC_{50} values for the more susceptible lake trout to the more tolerant largemouth bass was 33.8 to 63.0 p.p.m. The toxicity was not much greater in exposures of more than 24 hours.

Larger specimens of a species are usually more resistant to MS-222 than small ones.

The trout were slightly more resistant to the anesthetic at colder temperatures. Bluegills were more resistant at 17° than at 12° to 22° C.

Toxicity of MS-222 to rainbow trout was influenced very little by water hardness, but the fish reacted inconsistently in very soft water.

Among the survivors in partial-kill concentrations of MS-222 in 96-hour exposures,

the trout recovered from anesthesia more rapidly than other species.

Safety indexes based on the quotient of lethal and effective concentrations can be used to distinguish the margin between toxicity and efficacy.

SUMMARY

Toxicity of MS-222 to rainbow trout, brown trout, brook trout, lake trout, northern pike, bluegill, largemouth bass, and walleye of various sizes was determined in 15-, 30-, and 60-minute and 24-, 48- and 96-hour bioassays at selected temperatures. The 24-hour LC_{50} values for the more sensitive lake trout and more tolerant largemouth bass ranged from 33.8 to 63.0 p.p.m. and mortalities were not much greater in exposures which exceeded 24 hours.

Larger fish of a species are more resistant to the drug than small ones. Also, the trout were more tolerant at colder temperatures. Bluegills, on the other hand, were more tolerant at 17° than at 12° and 22° C.

The drug was tested against rainbow trout in waters with hardnesses of 10, 42, and 176 p.p.m. The fish reacted inconsistently in the softer water.

The recovery from anesthesia was evaluated in fish which survived partial-kill concentrations of MS-222 in 96-hour exposures. The trout recovered more rapidly than the other species, and posttreatment survival was good.

Safety indexes (LC_{50}/EC_{50}) and maximum safety indexes (LC_1/EC_{99}) were calculated from data obtained in 15-, 30-, and 60-minute exposures of rainbow trout to MS-222. They can be used to estimate the margin of safety between toxicity and efficacy. In one instance, the maximum safety index more clearly demonstrated the possible hazard of exposing small rainbow trout to the drug in soft water than did the safety index.

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13. Efficacy of MS-222 as an Anesthetic on Four Salmonids

By Richard A. Schoettger and Arnold M. Julin



United States Department of the Interior, Stewart L. Udall, *Secretary*
Stanley A. Cain, *Assistant Secretary for Fish and Wildlife and Parks*
Fish and Wildlife Service, Clarence F. Pautzke, *Commissioner*
Bureau of Sport Fisheries and Wildlife, John S. Gottschalk, *Director*
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EFFICACY OF MS-222 AS AN ANESTHETIC ON FOUR SALMONIDS

By Richard A. Schoettger and Arnold M. Julin, Fishery Biologists
Bureau of Sport Fisheries and Wildlife
La Crosse, Wis.

Abstract.--MS-222 was tested for its efficacy as an anesthetic for rainbow trout, brown trout, brook trout, and lake trout. Eighty to 135 p.p.m. of MS-222 anesthetized fish within 3 minutes at 7° to 17° C. The fish could be exposed for a total time of 4 to 12 minutes. Fifty to 60 p.p.m. induced a moderate rate of anesthesia which could be maintained for approximately 30 minutes. Sedation was produced within 15 minutes and maintained for 5 to 6 hours at 15 to 30 p.p.m. The efficacy of sedating concentrations appeared to decrease with time at 17° C. Lake trout required larger doses than the other salmonids for complete anesthesia but tolerated only short exposures. There was no relation between size of fish and efficacy of MS-222. Smaller fish occasionally had shorter exposure times. The drug was equally efficacious at pH values of 5.0, 7.0, and 8.5. Anesthetic solutions with a total hardness of 10 p.p.m. were less effective in anesthetizing rainbow trout than those containing 35 and 180 p.p.m. Individuals which were anesthetized in soft water recovered sooner.

MS-222 (tricaine methanesulfonate) was discovered by Maurice Sandoz during investigations to develop a synthetic substitute for cocaine. It was classified as a local anesthetic for use in human medicine. During the late 1920's, the drug also came into use as an anesthetic for poikilotherms including fish, salamanders and frogs (Bové, 1962).

The potential usefulness of MS-222 was not recognized widely in fisheries until Wood (1956) and Ball and Cowen (1959) reported on the carcinogenic properties of urethane, then a commonly used anesthetic for fish. An editorial comment accompanying Wood's report indicated that MS-222 might be a suitable substitute for urethane. Since then, the anesthetization of fish with MS-222 has become routine to facilitate the handling of both marine and freshwater species. The toxicity of the drug to fish was determined by Marking (1966). Walker and Schoettger (1966) measured its residues in various tissues of salmonids.

General guidelines for effective and safe use of MS-222 in fisheries are frequently misleading or lacking. The concentrations employed in fish culture, fisheries management and research were reviewed by Bové (1962), the manufacturer¹, Eisler and Backiel (1960), and Schoettger (1966). They differ widely depending on factors such as species and temperature. Unfortunately, potentially lethal concentrations may be required to achieve desired rates or depths of anesthesia. Thus, the durations of exposure are critical. If many fish are anesthetized at one time, some may become over exposed and die. Concentrations for selected rates of anesthesia, exposure times tolerated by the fish and factors affecting the drug's efficacy therefore

¹ Sandoz Pharmaceuticals: (No date) M.S. 222-Sandoz, the anesthetic of choice in work with cold-blooded animals, Sandoz Pharmaceuticals, Hanover, N.J., Technical Bulletin, 10 p.

required definition under controlled conditions.

Because of the widespread use of MS-222 in the culture and management of salmonids, we selected four representatives of the group for this study. The influences of temperature, water hardness, pH, repeated exposure and size of fish on the action of the anesthetic were evaluated.

METHODS AND MATERIALS

The efficacy of MS-222 was tested against rainbow trout, brown trout, brook trout, and lake trout of 2 to 6 inches and 7 to 12 inches (table 1). The test fish were held in well water at 12° C. and starved for two days prior to their placement in the anesthetic solutions. Twenty-four hours before an experiment, the fish were temperature conditioned in reconstituted water which was prepared according to methods described by Lennon and Walker (1964). Fish less than 4 inches long were tested in 15 liters of reconstituted water. Larger individuals were tested in 45 liters. Since preliminary tests failed to show that variable loading had any effect on efficacy, the loading levels were approximately 10 g./l. for the smaller fish, and up to 40 g./l. for the larger fish.

TABLE 1.--Trouts used in tests of MS-222

Source	
Rainbow trout (<i>Salmo gairdneri</i>).....	Manchester National Fish Hatchery, Manchester, Iowa.
Brown trout (<i>Salmo trutta</i>).....	Manchester National Fish Hatchery, Manchester, Iowa, and State Fish Hatchery at Lanesboro, Minn.
Brook trout (<i>Salvelinus fontinalis</i>)..	State Fish Hatcheries at Osceola, Wis., St. Croix Falls, Wis., and Lanesboro, Minn.
Lake trout (<i>Salvelinus namaycush</i>)...	Jordan River National Fish Hatchery, Charlevoix, Mich., and State Fish Hatchery at St. Croix Falls, Wis.

Initial trials with MS-222 demonstrated the need to establish: (1) general behavioral responses of fish to the anesthetic, and (2) criteria to evaluate effective concentrations.

The behavioral responses of fish treated with MS-222 are shown in table 2. Similar

TABLE 2.--General behavioral response of fish which characterize progressive levels of anesthesia

Sedation.....	Decreased reactivity to visual and vibrational stimuli; opercular and locomotor activity reduced slightly; darker in color.
Partial loss of equilibrium..	Loss of equilibrium in water current; increased opercular rate; swimming ability disrupted.
Total loss of equilibrium:	
Stage 1.....	Usually turn over; swimming ability persists; opercular rate rapid; react to vibrational stimuli.
Stage 2.....	Locomotion ceases; fin movement may continue; tactile response only to pressure on caudal fin or peduncle; opercular rate slowed.
Loss of reflex activity.....	Failure to respond to external stimuli, particularly pressure on caudal fin or peduncle; opercular rate slow and erratic.
Medullary collapse.....	Opercular activity ceases.

responses were reported by McFarland (1959 and 1960) and Klontz (1964). Recognition of these stages of anesthesia is essential to the selection of dosage rates, and to reduce the risk of excessive exposures.

The criteria established to determine the efficacy of MS-222, though somewhat arbitrary, were based on general considerations reported in the literature (table 3). It is difficult to predict concentrations of MS-222 which satisfy all requirements of an anesthetic; however, the levels associated with these criteria provide reference points which are adaptable to the specific needs for an anesthetic. The periods that fish tolerated exposure to MS-222 were determined by entry of the first and the last individual into medullary

Table 3.--Criteria for testing anesthetics

Use	Desired effect	Criteria for effective concentration
Handling: Fin clipping, stripping, etc.....	A. Rapid immobility Rapid recovery. Brief immersion time.	A. Concentration producing loss of reflex in all fish within 3 minutes. Total immersion time dependent on the onset of medullary collapse.
	B. Moderately rapid and sustained anesthesia.	B. Concentration producing total loss of equilibrium, stage 11, in all fish within 15 minutes. Fish recover after 15 to 30 minutes of immersion.
Transportation of fish.....	Reduced activity. Reduced oxygen consumption. Dispersal of fish in containers. Rapid recovery.	Concentration producing sedation within minutes and maintaining it for 5 to 6 hours.

collapse. The time of response is useful in estimating the numbers of fish which can be handled safely in a batch. In actual practice the more susceptible fish respond first to the anesthetic and are removed.

A series of concentrations of MS-222 were tested at a temperature of 12° C. and at pH 7.0. The levels giving reasonable anesthetization, holding and recovery times, and which produced minimum mortality were considered for further trials at 7°, 12° and 17° C., and at pH 5.0, 7.0, and 8.5. Temperature control was maintained by placing the test containers in water baths. The pH of some solutions was varied by the addition of sodium bicarbonate, or potassium acid phthalate and sodium hydroxide. The oxygen concentrations of the solutions were maintained close to saturation by aeration before, or during the experiments.

The metabolic rates of fish sedated with MS-222 were compared with those of unanesthetized fish. The studies were conducted in open and closed systems, and were designed to estimate the usefulness of the drug for suppressing the metabolism of fish in tanks, and in plastic bags.

Lake trout averaging 4 inches in length and 6.3 grams in weight were used in the open-system tests. Five individuals were placed in each of sixteen 1-gallon jars, each containing 2.5 liters of reconstituted water and the volume adjusted slightly to achieve a constant loading of 15 g./l. Eight test solutions at 12° C. contained sufficient MS-222 to produce sedation (20 p.p.m.), and were saturated with oxyben. At intervals, one container of fish from each experimental and control group was selected randomly and the oxygen was measured by the modified Winkler method. The tests continued until the fish showed signs of distress because of low oxygen.

In the closed-system trials, one or two 6-inch rainbow trout were weighed and sealed in 1-gallon jars that were completely filled with reconstituted water at 12° C. The solutions were saturated with oxygen at the beginning of the tests. The fish were sedated with 20 to 30 p.p.m. of MS-222 and the quantities of oxygen consumed by them were compared to controls

after 30 to 60 minutes. The values were corrected for the loading level in each jar.

The influence of water hardness on the efficacy of MS-222 was measured with 4-inch and 9-inch rainbow trout. The hardnesses of the various solutions were altered by adding different amounts of the mixed reconstituting salts. Preliminary tests were conducted in solutions of MS-222 which had hardnesses ranging from 17 to 350 p.p.m. as calcium carbonate. In subsequent trials a concentration of 100 p.p.m. of the drug was tested for efficacy in solutions with hardnesses of 10 and 180 p.p.m. These contained 5 and 75 p.p.m. of calcium ion, 1.5 and 7.0 p.p.m. of chloride ion, 9.5 and 116 p.p.m. total alkalinity, and had pH's of 7.14 and 8.20 respectively. They will later be referred to as relatively soft and hard waters. The trout were acclimated in both types of water for 24 to 48 hours preceding the experiments and were placed in well water for recovery.

The effect of repeated exposure of fish to MS-222 was tested by anesthetizing 12-inch rainbow trout and 6-inch lake trout daily in a concentration of 100 p.p.m. The time preceding the onset of medullary collapse, recovery time and survival were used as indices of sensitivity. The trials with rainbow trout continued for 21 days and were conducted in reconstituted water at 12° C. The fish were placed in well water for recovery. In similar experiments lake trout received 12 treatments. Their sensitivity to the anesthetic was compared periodically with untreated individuals. The fish were anesthetized and recovered in well water. Both species were tested in groups of 10 and fed their normal ration throughout the trials.

RESULTS AND DISCUSSION

The efficacy of MS-222 for inducing rapid and moderately rapid anesthesia and sedation in two size groups of salmonids was tested at 7° to 17° C. and pH 5.0 to 8.5. The efficacy of the drug appeared unaltered by pH and these data were grouped, without differentiation, with those for size of fish and temperature.

Rapid anesthesia

Rainbow trout were anesthetized to loss of reflex within 3 minutes in solutions containing 100 p.p.m. of MS-222 (table 4). Fish in both size ranges were equally sensitive to the drug and in contrast to other salmonids, there appeared to be less effect of temperature on efficacy. The fish withstood exposure for approximately 5.5 to 12 minutes. The durations were somewhat shorter at higher temperatures and with smaller fish.

A concentration of 80 p.p.m. was 100-percent effective on brown trout at 17⁰, however, the concentration was raised to 100 p.p.m. at 7⁰ and 12⁰ C. to achieve efficacies of 86.6 to 100 percent (table 4). Though a weaker solution was used at 17⁰, the fish reached medullary collapse and recovered sooner than at 7⁰ and 12⁰. This suggests a greater metabolism of MS-222 at higher temperatures.

Brook trout and lake trout were effectively anesthetized by 100 p.p.m. of MS-222 at 17⁰

TABLE 4.--Concentrations of MS-222 producing rapid anesthesia in four salmonids at three temperatures.

Species and concentration	Temper- ature (C.)	Size of fish inches	Fish in loss of--				Mean range of exposure times (minutes)		Safe exposure index ¹	Recovery	
			Equilibrium, stage 11, with 2 minutes		Reflex within 3 minutes		First fish	Last fish		Mean time range minutes	Survival (percent)
			Number	Percent	Number	Percent					
Rainbow trout:											
100 p.p.m.....	7°	2-6	60/60	100.0	60/60	100.0	6.6	8.1	2.2	4.0-11.0	98.4
Do.....	7°	7-12	20/20	100.0	20/20	100.0	6.8	11.4	2.3	5.1-11.8	100.0
Do.....	12°	2-6	173/173	100.0	170/173	98.3	5.5	7.0	1.8	2.8- 8.2	93.1
Do.....	12°	7-12	95/95	100.0	95/95	100.0	8.0	11.9	2.7	4.2- 8.6	100.0
Do.....	17°	7-12	20/20	100.0	20/20	100.0	5.5	6.9	1.8	4.3- 9.0	93.3
Brown trout:											
80 p.p.m.....	12°	2-6	20/20	100.0	13/20	65.0	6.9	13.3	2.3	3.3- 6.6	100.0
Do.....	17°	2-6	5/5	100.0	5/5	100.0	5.3	7.3	1.8	6.0- 6.3	40.0
Do.....	17°	7-12	20/20	100.0	20/20	100.0	4.6	7.0	1.5	3.9- 8.2	86.6
90 p.p.m.....	7°	2-6	13/15	86.6	9/15	60.0	9.4	10.4	3.1	5.8-10.1	100.0
Do.....	12°	2-6	40/40	100.0	39/40	97.5	5.9	8.3	2.0	3.8- 7.6	100.0
Do.....	17°	7-12	8/8	100.0	8/8	100.0	4.5	6.1	1.5	4.8-17.0	75.0
100 p.p.m.....	7°	2-6	30/30	100.0	26/30	86.6	7.9	9.4	2.6	6.4-18.5	100.0
Do.....	7°	7-12	34/35	97.1	32/35	91.4	9.5	12.1	3.2	7.3-11.0	96.6
Do.....	12°	2-6	60/60	100.0	60/60	100.0	6.1	8.1	2.0	4.1- 9.6	100.0
Do.....	12°	7-12	55/55	100.0	53/55	96.3	7.9	11.0	2.6	4.9-13.3	98.1
Brook trout:											
100 p.p.m.....	12°	2-6	70/70	100.0	43/60	71.6	5.0	--	1.7	2.0- 6.4	100.0
Do.....	17°	7-12	20/20	100.0	20/20	100.0	4.2	5.4	1.4	5.1- 8.9	93.3
110 p.p.m.....	12°	7-12	30/30	100.0	30/30	100.0	6.5	8.7	2.2	4.4- 7.6	100.0
120 p.p.m.....	7°	7-12	24/25	96.0	24/25	96.0	8.8	11.4	2.9	4.1- 7.7	100.0
Lake trout:											
100 p.p.m.....	7°	2-6	38/120	31.6	10/120	8.3	6.9	8.5	2.3	5.5-19.2	85.8
Do.....	12°	2-6	101/120	84.1	71/120	59.2	4.7	5.4	1.6	4.1-15.8	91.7
Do.....	17°	7-12	25/25	100.0	25/25	100.0	3.9	4.7	1.3	3.4- 5.3	100.0
110 p.p.m.....	12°	7-12	30/30	100.0	29/30	96.7	5.0	6.3	1.7	3.5- 9.1	100.0
135 p.p.m.....	7°	7-12	18/25	72.0	20/25	80.0	5.6	6.5	1.9	4.4- 7.1	100.0

¹ Index obtained by dividing the time for the first fish to reach medullary collapse by the time (3 minutes) for fish to reach loss of reflex.

and by 110 p.p.m. at 12⁰ (table 4). The dosage was raised to 120 p.p.m. for brook trout, and 135 p.p.m. for lake trout at 7⁰. The concentrations for lake trout are similar to those used at a national fish hatchery² in Michigan.

In general, the chars were more resistant to MS-222 at 7⁰ and 12⁰ than rainbow trout or brown trout. This may indicate a lower, and possibly narrower, range of optimum tem-

peratures for the former species. The exposures for brook trout ranged from 4.2 minutes at 17⁰ to 11.4 minutes at 7⁰. The values for lake trout, at the same temperatures, were 3.9 and 6.5, and they reflect a greater sensitivity than the other salmonids to prolonged anesthetization. Also, the lake trout died quickly when they were not removed from the anesthetic soon after the cessation of respiration. This was not as critical with the other species and suggests that the respiratory and cardiac centers of lake trout are inhibited at a similar rate by the drug.

² Personal communication from George Drake, Hatchery Manager, Pendills Creek National Fish Hatchery, Brimley, Mich.

The progress of anesthesia in the various salmonids slowed, and exposure times lengthened with decreasing temperature. Although lower dosages were used at 17° C., with the exception of rainbow trout, it was necessary to remove the fish from the anesthetic solution sooner than at 7°. The reduced efficacy at relatively low temperatures is in accord with the results of Meister and Ritzi (1958). We concluded that rapid anesthesia is more safely induced and maintained at lower temperatures.

The drug appeared to be similarly effective on both size ranges of rainbow trout and brown trout; however, larger fish withstood longer anesthesia. Brook trout and lake trout of smaller sizes were not tested at the concentrations used for larger specimens. The trouts usually recovered from deep anesthesia within 3 to 15 minutes. There were no consistent relations between recovery time and temperature, size of fish, or total exposure.

MS-222 is capable of inducing rapid anesthesia which makes it useful in spawning, marking, and measuring fish, and in immobilizing specimens for various physiological investigations (Nelson, 1953; Butler, 1957; Parkhurst and Smith, 1957; Schiffman and Fromm, 1959; Crawford and Hulsey, 1963; Black and Connor, 1964). Its relatively high narcotic potency to fish is related to its molecular weight (McFarland, 1959).

The rate of anesthetization with MS-222 can be controlled by the dosage. According to the literature, rapid results have been achieved with concentrations ranging from approximately 80 to 1,000 p.p.m. The action of the drug is reversible at these concentrations provided exposure time is manipulated to minimize mortality. We observed that anesthetized trout always recovered when they were transferred to fresh water before cessation of respiratory activity. Rodman (1963) found that anesthesia of white sturgeon was not reversible when the fish were exposed to a concentration of 1:40,000 for 30 to 48 hours. Marking (1966) maintained rainbow trout under anesthesia with MS-222 for 96 hours. They recovered quickly in fresh water and appeared to be healthy seven days later.

Our results, and those of other investigators, demonstrate that relatively high concentrations of MS-222 are effective and safe for the rapid anesthetization of trout. The dependence of survival on exposure time suggests that an index for the safe exposure of fish to MS-222 can be obtained from a quotient of the time for medullary collapse and that for anesthesia. Indexes for the various species and sizes of fish were calculated for temperatures of 7°, 12°, and 17° C. (table 4). They are biased in favor of greater safety. High indexes may be calculated from times for shallower anesthesia and longer exposure. Usually, the indexes were greater than 1.5. The values of 12° and 17° were relatively lower than those at 7° and indicated a greater hazard in anesthetizing fish with MS-222 at higher temperatures. Brook trout and lake trout, for example, had respective indices of 1.4 and 1.3 at 17°, whereas the values were 2.9 and 1.9 at 7° C.

Effects of water hardness.--In preliminary studies, we found that the water harnesses from 17 to 350 p.p.m. had little influence on the anesthetic properties of MS-222 against rainbow trout.

Phillips et al. (1955) established the importance of the calcium ion in the metabolism and osmoregulation of brook trout. Calcium concentrations of 5 p.p.m. increased metabolism, presumably in active opposition to the osmotic inflow of water. We anticipated that solutions with a hardness of 10 p.p.m. and a calcium ion level of 5 p.p.m. might introduce an osmotic stress which would alter the anesthetic action of MS-222 against rainbow trout. The results of trials in solutions with hardnesses of 10 p.p.m. and 180 p.p.m. are shown in table 5. In soft water, the times to

TABLE 5.--Effect of water hardness on the efficacy of 100 p.p.m. of MS-222 against 9-inch rainbow trout at 12° C.

	Mean time in minutes to--					
	Loss of reflex in water with hardness of--		Medullary collapse in water with hardness of--		Recovery in water with hardness of--	
	10 p.p.m.	180 p.p.m.	10 p.p.m.	180 p.p.m.	10 p.p.m.	180 p.p.m.
Six tests (80 fish).....	7.98	2.43	16.80	8.10	4.68	6.67
Standard deviation.....	1.98	0.29	3.61	1.94	1.57	2.22
Differences between means	5.55		8.70		1.99	
Calculated "t",.....	17.56*		13.41*		4.98*	

*Significant at the 0.01 level.

reach complete anesthesia and durations of exposure were double those for hard water, and recovery times were shorter. The mean differences between soft and hard water were statistically significant at the 0.01 level. Onkst et al. (1957) observed that induction of anesthesia in guppies with pentobarbital was inhibited when the fish were acclimated and tested in a calcium deficient medium. Marking (1966) observed that higher concentrations of MS-222 were required to anesthetize rainbow trout in soft water.

The results in hard water agree closely with those in table 4 which were determined in waters with a hardness of 35 p.p.m. The data in tables 4 and 5 indicate that there is a threshold of ions below which the efficacy of MS-222 is partially inhibited. Ions other than calcium may also contribute to this effect.

The reduced effectiveness of MS-222 in soft water is not due to lower uptake of the drug. Greater amounts of MS-222 were found in the muscle tissues of fish anesthetized in soft water than in those treated in hard water (Walker and Schoettger, 1966). The longer exposures of trout in soft water, and increases in their metabolism to maintain osmotic equilibrium, may account for the higher levels of MS-222 in these fish.

We observed that the rates of anesthetization of fish to total loss of equilibrium, stage 1, were similar in both soft and hard water. The later stages of anesthesia, however, occurred slowly in soft water. Using McFarland's (1959) suggested classification of anesthesia in fishes, there appeared to be no change in the depressive action of MS-222 on the telencephalon, sensory areas of the tectum and tegmentum, and midbrain and thalamus of fish treated in soft water. Depressions of the motor nuclei in the medulla, the spinal reflex arcs, and of respiratory and cardiac centers are responsible for deeper anesthesia. It is possible that in these areas the activity of the anesthetic was antagonized by osmotic disturbances of the potassium-calcium balance in the nerve cells. Also, considering the results of Phillips et al. (1955) and Walker and Schoettger (1966), the rate of absorption and deactivation of MS-222 may have increased

along with higher metabolic rates of fish in soft water.

Phillips et al. (1957) showed that the narcotization of brook trout with MS-222 increased their absorption of radioactive cobalt. They suggested that there may be an adjustment of the osmotic processes of narcotized fish. According to Quastel (1963) anesthetics have a twofold mechanism of action on the respiration and metabolism of brain tissue: (1) effects on the cationic equilibrium of the cell membrane, and (2) the anesthetic may affect the oxidation of diphosphopyridine nucleotide within the cell. The first mechanism may occur without the second. Changes in ionic balance such as an increase in cellular potassium or a decrease in calcium stimulate respiration of the nerve cell. An anesthetic may deactivate carriers at the cell membrane which are responsible for cation movements, and have an effect similar to that of a respiratory inhibitor acting on the mitochondria. It is not known whether the action of MS-222 on the central nervous system of fish is in accord with this scheme for other anesthetics.

Effects of repeated anesthetization.--The daily anesthetization of 10 rainbow trout over 21 days, and 10 lake trout over 12 days with 100 p.p.m. of MS-222 produced no progressive increase, or decrease in their tolerance to the drug. The tolerances of lake trout were compared periodically with those of previously untreated individuals. The repeatedly exposed fish withstood anesthesia for approximately 1.0 to 1.5 minutes longer than the control group. The higher tolerance apparently originated after the first treatment. Recovery time and survival were not affected by repeated exposure. Klontz (1964) reported a slight increase in tolerance of fish anesthetized repeatedly with MS-222 which was compensated by increasing the concentration.

Moderately rapid anesthesia

A moderate rate of anesthesia is useful in situations where fish must be handled over a longer time, but where rapid immobilization is not essential. For example, MS-222 is used to anesthetize fish during surgery and hematological studies (Robertson, 1958; Smith and Bell, 1964).

We determined that concentrations of 50 to 60 p.p.m. anesthetized the four salmonids to total loss of equilibrium, stage 2, within 15 minutes (table 6). The fish usually could remain in the anesthetic for 30 minutes.

A concentration of 60 p.p.m. was effective in anesthetizing rainbow trout within 15 minutes, but attempts to maintain them under anesthesia for 30 minutes resulted in mortalities of approximately 8 to 25 percent. More individuals died at 12° than at 7° C. The anesthetic was almost as effective at 50 to 55 p.p.m., and mortality was less.

MS-222 was effective on brown trout at 50 p.p.m., and only 1 fish of 170 failed to recover (table 6).

The chars were relatively less sensitive to MS-222 than the other fish and a concentration of 60 p.p.m. was required to induce anesthesia within 15 minutes. The data indicate that there was a slight reduction in efficacy of the drug at 12° C., which suggests some deterioration, or increased metabolism, of MS-222 in higher temperatures. Although lake trout appeared to be resistant, some did not tolerate exposures to 60 p.p.m. longer than 15 minutes. A concentration of 50 p.p.m. lengthened the immersion time, but reduced the initial speed of anesthetization.

All of the brook trout recovered from anesthesia, but mortalities of lake trout ranged

from 15 to 35 percent and were greater at 12° than at 7° C. The mortalities were probably related to our inability to pinpoint the onset of medullary collapse. The duration of the loss of reflex in lake trout is very brief.

The recovery of fish in these tests usually occurred within 1 to 15 minutes.

Sedation

MS-222 can be used to produce sedation in fish. Solutions containing 20 p.p.m. of the drug were, with several exceptions, 80- to 100-percent effective in sedating rainbow trout (table 7). Brown trout were sedated by 15 p.p.m. Thirty p.p.m. were required for larger rainbow trout at 17° and for brook trout at 7° C. The trout usually recovered within 1 minute after they were transferred to fresh water. Concentrations similar to these have been used to sedate white sturgeon, sockeye salmon, cutthroat trout, rainbow trout, and bluegill (Rodman, 1963; Meehan and Revet, 1962; Thompson, 1959; Dollar, 1963; Gebhard, 1965; Webb, 1958). Lamarque (1964) recommended dosages of 20 to 100 p.p.m., depending on temperature, for the preliminary anesthesia of fish before they are placed in plastic bags for shipment. He suggested 10 p.p.m. for tranquilization of fish in hauling tanks.

McFarland (1959) stated that pH may influence the absorption of anesthetics into the gills of fish. Our results, however, showed no

TABLE 6.--Concentrations of MS-222 producing moderately rapid anesthesia in fish to total loss of equilibrium, stage 2, within 15 minutes

Species and concentration	Temperature (C.)	Size of fish (inches)	Fish in anesthesia		Exposure time (minutes)	Recovery	
			Number	Percent		Mean time range (minutes)	Survival (percent)
Rainbow trout:							
50 p.p.m.....	7°	2-6	43/45	95.5	15->30	1.40- 4.00	100.0
Do.....	12°	2-6	182/210	86.7	>30	1.59-17.54	98.9
55 p.p.m.....	12°	2-6	20/20	100.0	30	2.75-11.25	100.0
60 p.p.m.....	7°	2-6	134/135	99.2	15->30	3.40- 9.20	91.8
Do.....	12°	2-6	85/85	100.0	15- 30	2.20-16.60	75.2
Brown trout:							
50 p.p.m.....	7°	2-6	30/30	100.0	30	4.40- 6.80	100.0
Do.....	7°	7-12	10/10	100.0	30	6.20-52.00	90.0
Do.....	12°	2-6	130/130	100.0	30	2.30- 4.60	100.0
Brook trout:							
60 p.p.m.....	7°	2-6	40/40	100.0	20- 30	3.20- 8.00	100.0
Do.....	12°	2-6	50/60	83.3	30	3.20- 5.70	100.0
Lake trout:							
40 p.p.m.....	12°	2-6	0/10	0.0	30	1.00- 1.50	100.0
50 p.p.m.....	7°	2-6	72/120	60.0	30	3.41-13.57	83.3
Do.....	12°	2-6	47/140	33.5	15- 30	2.23-15.23	65.8
60 p.p.m.....	7°	2-6	133/140	95.0	15- 30	3.70-13.10	85.0
Do.....	12°	2-6	84/120	74.0	15- 30	1.60-21.80	65.0

TABLE 7.--Concentrations of MS-222 producing sedation in four salmonids

Species and concentration	Temperature (C.)	Size range (inches)	Fish in sedation at--				Behavior ¹ of fish not in sedation at--	
			15 minutes		5-6 hours		15 minutes	5-6 hours
			Number	Percent	Number	Percent		
Rainbow trout:								
20 p.p.m.....	7°	2-6	132/135	97.8	129/135	95.6	>	>
Do.....	7°	7-12	80/120	66.6	78/120	65.0	>	>
Do.....	12°	2-6	175/177	98.8	177/177	100.0	>	>
Do.....	12°	7-12	81/90	90.0	84/90	93.3	>	>
Do.....	17°	7-12	32/40	80.0	22/40	55.0	<	<
30 p.p.m.....	12°	7-12	20/20	100.0	16/20	80.0	>	>
Do.....	17°	7-12	14/20	70.0	16/20	80.0	>	>
Brown trout:								
15 p.p.m.....	7°	2-6	20/35	57.1	33/35	94.3	>	>
Do.....	7°	7-12	45/50	90.0	49/50	98.0	>	>
Do.....	12°	2-6	28/30	93.3	30/30	100.0	>	>
Do.....	17°	7-12	20/20	100.0	20/20	100.0	>	>
20 p.p.m.....	7°	2-6	9/36	25.0	23/36	63.9	>	>
Do.....	12°	2-6	21/72	29.2	70/72	97.2	>	>
Do.....	12°	7-12	17/20	85.0	20/20	100.0	>	>
Brook trout:								
20 p.p.m.....	7°	2-6	10/10	100.0	5/10	50.0		<
Do.....	12°	7-12	20/20	100.0	20/20	100.0		
Do.....	17°	7-12	20/20	100.0	20/20	100.0		
30 p.p.m.....	7°	2-6	57/60	95.0	50/60	83.3	>	>
Do.....	7°	7-12	20/20	100.0	19/20	95.0	>	>
Do.....	12°	2-6	29/30	96.7	24/30	80.0	>	>
Do.....	12°	7-12	9/20	45.0	20/20	100.0	>	>
Do.....	17°	7-12	12/20	60.0	15/20	75.0	>	< >
Lake trout:								
20 p.p.m.....	7°	2-6	179/180	99.5	146/150	97.3	>	>
Do.....	7°	7-12	17/20	85.0	17/20	85.0	<	<
Do.....	12°	2-6	280/280	100.0	185/220	84.0		<
Do.....	12°	7-12	20/20	100.0	20/20	100.0		
Do.....	17°	7-12	18/20	90.0	16/20	80.0	<	<

¹ > =deeper anesthesia; < = similar to controls.

important effects of pH 5.0 to 8.5 on the sedating properties of MS-222.

Sedation is not a static stage of anesthesia. The stage shifts toward recovery, or deeper anesthesia, according to the concentration of anesthetic, temperature, and, in some instances, the size of the fish. The individuals in nearly 70 percent of the trials conducted at 17° C. showed signs of recovery after 6 hours. The trend was never toward deeper anesthesia in tests at this temperature. The shift at 7° and 12° was toward recovery in 40 percent of the tests, and toward deeper anesthesia in 20 percent. Sedation persisted in the remaining 40 percent. The reduced effectiveness of the drug at relatively higher temperatures is probably related to its deterioration, increased metabolism by the fish, or both. More conclusive evidence regarding the temperature dependence of MS-222 was obtained by McFarland (1959, 1960). His observations on the behavior and metabolism of anesthetized California killifish demonstrate that the drug was an effective sedative at 18° to 21° C., but not at 24° to 27° C. Sedation at the lower temperature range was not maintained beyond 6 to 8 hours.

One of the principal uses of anesthetics in the transportation of fish is to control metabolism. Baudin (1932a, 1932b), McFarland (1959), and Blahm (1961) reported that MS-222 reduces the rate of oxygen consumption in fish. We observed that the drug reduced the oxygen consumption of rainbow trout in a closed system by approximately 30 percent (table 8). Statistical analyses indicated that the reduction was significant at the 0.05 but not at the 0.01 level. In contrast, the anesthetic failed to reduce significantly the oxygen consumption rate of lake trout in open-system tests (table 9). This may have been the result of the differential diffusion of oxygen into those solutions with greater oxygen gradients. Thus, there is little advantage in using MS-222 in open systems to reduce oxygen consumption. On the other hand, the fish in these experiments were not purposely stimulated to higher levels of activity. Fry (1957) demonstrated that the oxygen uptake of stimulated fish may be four to five times the basal metabolic rate. The drug may be efficacious in open systems by lowering metabolic rates of this magnitude. McFarland (1960) suggested the pre-sedation of fish for several hours to lower their metabolic rates before transport.

TABLE 8.--Oxygen consumption of 6-inch rainbow trout sedated with MS-222 in closed systems at 12°.

Test	Rates of oxygen consumption (mg.O ₂ /kg. body wt./hr.)	
	Sedated fish	Control fish
No. 1.....	162.4	162.4
No. 2.....	162.7	162.7
No. 3.....	162.6	162.6
No. 4.....	168.7	168.7
No. 5.....	161.2	161.2
No. 6.....	171.2	171.2
No. 7.....	162.4	162.4
No. 8.....	178.3	178.3
No. 9.....	161.2	161.2
Mean.....	162.5	162.5
Standard deviation.....	57.7	57.6
Difference between mean rates....	67.7	
Calculated "t".....	2.63*	

*Significant at the 0.05, but not at the 0.01 level.

The reports on the benefits of sedating fish with MS-222 during transport are contradictory (Webb, 1958; Martin and Scott, 1959; Thompson, 1959; Meehan and Revet, 1962; Dollar, 1963; Rodman, 1963; Gebhards, 1965). The drug has been used more successfully at low, than at high temperatures, and it may be instrumental in reducing injuries to fish because of hyperactivity (Gossington, 1957; McFarland, 1960; Collins and Hulsey, 1963).

Use of MS-222 as a spawning aid

Anesthetization of gravid rainbow trout and brook trout with MS-222 was observed at three hatcheries. The rainbow trout were treated at national fish hatcheries at Genoa, Wis., and Manchester, Iowa, and the brook trout were exposed at the state fish hatchery at Osceola, Wis. The concentrations of drug were those normally used at the hatcheries

during the collection of eggs and sperm. The observations are listed in table 10.

The amounts of MS-222 used to anesthetize the sexually mature, adult rainbow trout were similar to those producing rapid and moderately rapid anesthesia in the efficacy tests (table 4 and 6). A concentration of 58 p.p.m. produced loss of reflex in about 6 minutes, and it was as effective on fish weighing 8 pounds as on smaller individuals. The fish remained in this concentration for 6 to 28 minutes, and all recovered in 3 to 5 minutes. The desirable feature of this dosage was that a greater number of fish could be anesthetized over a longer period than would have been possible using higher dosages. Twenty fish were exposed in one trial and some remained in the anesthetic for nearly 30 minutes. They were not completely anesthetized and reacted slightly when handled. The more susceptible individuals were spawned within 5 minutes after contact with the anesthetic.

The fish exposed to 100 p.p.m. reached loss of reflex in 1.25 and 3.25 minutes. The duration of exposure was 2 to 7 minutes, and all recovered in 1 to 3 minutes.

The 166 p.p.m. of MS-222 used for male brook trout were higher than those employed in our efficacy tests (table 4). They were exposed for 4.25 to 7.5 minutes and all recovered in 7 to 9 minutes. The hatchery personnel reported that as many as 150 fish are anesthetized in one solution of MS-222 before

TABLE 9.--The oxygen consumption of 4-inch lake trout sedated with MS-222 in open systems at 12° C.

Elapsed time	Sedated fish			Control fish		
	Concentration of oxygen (p.p.m.)	Consumption of oxygen		Concentration of oxygen (p.p.m.)	Consumption of oxygen	
		p.p.m./hr.	mg.O ₂ /kg. body wt./hr.		p.p.m./hr.	mg.O ₂ /kg. body wt./hr.
0 hours.....	9.8	--	--	9.4	--	--
1.25 hours.....	8.3	1.2	80.0	6.8	2.1	140.0
1.5 hours.....	7.0	1.9	126.7	6.4	2.0	133.3
2 hours.....	6.5	1.7	113.3	6.2	1.6	106.7
2.5 hours.....	5.6	1.7	113.3	4.7	1.9	126.7
3 hours.....	5.2	1.5	100.0	4.6	1.6	106.7
3.5 hours.....	5.0	1.4	93.3	4.4	1.4	93.3
4 hours.....	4.2	1.4	93.3	4.0	1.4	93.3
4.5 hours.....	3.6	1.4	93.3	3.0	1.4	93.3
Mean.....	1.5	101.7	1.7	111.6
Standard deviation.....	15.0	19.1
Difference between mean rates.....	9.9					
Calculated "t".....	1.1					

TABLE 10.--Observations on use of MS-222 in anesthetizing rainbow trout and brook trout during spawning operations

Species and hatchery	Concentration (p.p.m.)	Temperature (F.)	Mean weight per fish (pounds)	Number of fish per test	Number of tests	Sex	Time (minutes) to reach loss of--		Range of exposures (minutes)	Recovery in fresh water	
							Equilibrium, stage 2	Reflex		Percent	Time (minutes)
Rainbow trout:											
Manchester, Ia.....	58	54°	7.0	10	1	F	4.00	N.R. ¹	6.00- 8.00	100	3.00
Do.....	58	54°	7.0	6	1	F	5.00	N.R. ¹	6.00- 8.00	100	4.00
Do.....	58	54°	7.0	11	1	F	5.50	N.R. ¹	6.00- 8.00	100	3.00
Do.....	58	54°	1.0	20	1	M	3.25	6.00	7.00-28.00	100	3.00-5.00
Do.....	58	54°	1.0	15	1	M	5.50	N.R. ¹	N.R. ¹	100	N.R. ¹
Genoa, Wis.....	100	51.5°	0.9	2	3	F	1.00-1.50	1.50-3.25	2.00- 5.00	100	2.50-4.50
Do.....	100	51.5°	1.0	2	6	M	1.00-2.00	1.25-3.00	3.50- 7.00	100	1.00-3.00
Brook trout:											
Osceola, Wis.....	110	48°	1.6	2	2	F	0.75	1.25-1.50	2.00- 2.50	100	2.00
Do.....	110	48°	1.6	7	2	F	1.00-1.50	2.25-3.25	3.75- 5.75	100	8.00-9.75
Do.....	166	48°	1.4	2	2	M	1.00	1.25-2.00	4.25- 5.00	100	7.00-8.00
Do.....	166	48°	1.4	7	2	M	1.00	2.00-2.25	4.00- 7.50	100	8.00-9.00

¹ Not recorded.

its effectiveness is greatly reduced. Meister and Ritzi (1958) reported that from 22 to 35 pounds of brook trout, 36 to 86 pounds of Atlantic salmon, or 93 pounds of lake trout could be anesthetized per gram of MS-222.

The use of MS-222 during spawning speeds the operation and reduces the mortality of fish due to forceful handling. The anesthetic apparently has no adverse effects on the survival of eggs or parents (Parkhurst and Smith, 1957; Crawford and Hulsey, 1963). The exposed fish, however, are usually dipped into clean water before stripping to prevent the anesthetic from contacting reproductive products (Allison, 1961).

Other observations on MS-222

Minor deviations in the efficacy of MS-222 were observed from time to time. We believe that they were partially related to variations in the susceptibilities of individuals and groups of fish, or combinations of unknown factors. In any case, it is advisable to conduct preliminary bioassays of anesthetic solutions with several individuals from the stocks of fish which are to be narcotized. The precaution was recommended also by other investigators including Klontz (1964), Lamarque (1964) and Bell (1964). Observations on the depths of anesthesia and exposure times can be used to determine the numbers of fish which can be anesthetized safely.

CONCLUSIONS

Anesthetization of rainbow trout, brown trout, brook trout, and lake trout with MS-222

produced behavioral responses which were similar to those reported for other anesthetics and species of fish.

Concentrations of MS-222 which produced rapid anesthesia in the four salmonids ranged from 80 to 135 p.p.m. Levels of 50 to 60 p.p.m. induced moderately rapid anesthesia, and 15 to 30 p.p.m. were effective for sedation.

The action of MS-222 was reversible when the fish were removed from anesthetic solutions prior to the cessation of respiratory activity (medullary collapse). The retention of fish in solutions which are effective for rapid and moderately rapid anesthesia may result in mortality depending on the duration of exposure.

Brook trout and lake trout were more resistant to MS-222 at 7° and 12° C. than the other salmonids. Lake trout could withstand only short exposures and they died quickly after entering medullary collapse.

Effective concentrations and durations of exposure, particularly for rapid anesthesia, were inversely related to temperature. The rapid anesthetization of fish is more safely induced at lower temperatures.

There was little relation between size of fish and the efficacy of MS-222; however, the smaller fish occasionally had shorter exposure times.

The anesthetic solutions which contained 10 p.p.m. total hardness were less effective in narcotizing rainbow trout than those with hardnesses of 35 and 180 p.p.m. The fish anesthetized in soft water recovered sooner.

The trout which were repeatedly anesthetized with MS-222 were slightly more tolerant than previously unexposed individuals. The tolerance, however, did not increase with repeated treatments.

Sedation was not a static stage of anesthesia, but shifted toward recovery, or deeper anesthesia depending on the concentration of anesthetic and temperature.

MS-222 did not reduce the rate of oxygen consumption of lake trout in open systems. The consumption rate of rainbow trout in closed systems was reduced by approximately 30 percent. The efficacy of MS-222 was not altered to a measurable degree by pH 5.0 to 8.5.

Our results and those of other investigators demonstrate that MS-222 is effective and safe for inducing various stages of anesthesia in the four salmonids.

SUMMARY

Investigations were made to determine the concentrations of MS-222 which produce rapid, moderately rapid, and sedating anesthesia in rainbow trout, brown trout, brook trout, and lake trout. Eighty to 135 p.p.m. of MS-222 rapidly anesthetized the fish within 3 minutes at temperatures of 7° to 17° C. The fish could be exposed to the anesthetic for approximately 4 to 12 minutes. The effective concentrations and exposure times varied with species and temperature. Fifty to 60 p.p.m. induced a moderate rate of anesthesia which could be maintained for approximately 30 minutes. Sedation was produced within 15 minutes and maintained for 5 to 6 hours at 15 to 30 p.p.m. The efficacy of sedating concentrations appeared to decrease with time at 17° C. Lake trout required higher dosages of MS-222 than other salmonids for complete anesthesia, but they tolerated only short exposures.

There was no relation between size of fish and efficacy of MS-222. Smaller fish, however, occasionally had shorter exposure times. The efficacy of the drug was not influenced by pH 5.0 to 8.5.

Anesthetic solutions with a total hardness of 10 p.p.m. were less effective in anesthetizing rainbow trout than those containing 35 to 180 p.p.m. Individuals which were anesthetized in soft water recovered sooner.

Repeatedly anesthetized trout were slightly more tolerant than previously unexposed fish. Tolerance did not increase with repeated treatments.

Sedating concentrations of MS-222 did not lower the rate of oxygen consumption of lake trout in open systems. In closed systems, the consumption rate of rainbow trout was reduced approximately 30 percent.

We concluded that MS-222 is an effective and safe anesthetic for inducing anesthesia in the four salmonids. The relative safety of the drug was greater at lower temperatures. Our results and those of other investigators indicate that preliminary bioassays of anesthetic solutions are advisable to determine the desired rates of anesthesia and exposure times for specific conditions and lots of fish. Close observance on stages of anesthesia is essential to prevent excessive exposure of the fish at high concentrations.

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14. Method for Determining MS-222 Residues in Fish

By Charles R. Walker and Richard A. Schoettger



United States Department of the Interior, Stewart L. Udall, *Secretary*
Stanley A. Cain, *Assistant Secretary for Fish and Wildlife and Parks*
Fish and Wildlife Service, Clarence F. Pautzke, *Commissioner*
Bureau of Sport Fisheries and Wildlife, John S. Gottschalk, *Director*
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METHOD FOR DETERMINING MS-222 RESIDUES IN FISH

By Charles R. Walker, Chemist, and Richard A. Schoettger, Fishery Biologist
Bureau of Sport Fisheries and Wildlife
La Crosse, Wisconsin

Abstract.--MS-222 is a primary aromatic amine. Its diazonium salt reacts with the Bratton-Marshall reagent (N-1-naphthylethylenediamine dihydrochloride) to form a wine-red azo dye with a maximum absorbance at 545 millimicrons. The diazotization reagent had to be modified and the reaction time extended to obtain a measurable yield of the diazonium salt. The regression of absorbance values versus concentrations of MS-222 which ranged from 0 to 7.5 p.p.m. had a slope of 1 and adhered to Beer's law. MS-222 was spiked into samples of blood, muscle, liver, and kidney from rainbow trout, and excellent recoveries were measured by the analytical method. The residues had to be distinguished from a background of primary aromatic amines which varied in each tissue, between individual specimens and with each lot of fish. Backgrounds were higher in liver and kidney, and residues of MS-222 were more difficult to differentiate. The method was particularly effective in determining residues of the drug in muscle and blood of trout.

MS-222 (tricaine methanesulfonate) is widely used as a fish anesthetic. Residues which might occur in fish flesh following use of the drug must be ascertained for safety of human consumers. Development of adequate information on residues required a method capable of measuring finite concentrations in the tissues.

A primary consideration in the acceptance of any analytical method for residues is the capacity to detect them at levels related to their toxicity to mammals. A contract study done for us by the Wisconsin Alumni Research Foundation in March 1965 indicated that the oral LD₅₀ of MS-222 for Sprague-Dawley strain laboratory rats is between 5 and 10 grams per kilogram of body. Thus, the drug is relatively nontoxic to mammals according to the standards of the American Industrial Hygiene Association (Spector, 1956). An analytical procedure capable of defining residues in parts per million should be adequate for all practical purposes.

Friddle and Snieszko (1950), using the Bratton-Marshall (1939) method, found that MS-222 caused an error in their evaluation of sulfa residues in anesthetized brook trout. We believe this can be explained by the fact that MS-222 is a primary aromatic amine and reacts readily in the colorimetric procedure. We chose to investigate the applicability of this colorimetric reaction to determinations of MS-222 in fish tissues.

METHODS AND MATERIALS

Experiments on operating parameters

Diazotization of the m-aminoethylbenzoate constituent of the MS-222 salt was accomplished by modification of the procedure described by Bratton and Marshall (1939). The conditions for formation of the diazonium ion and the subsequent reaction with the coupling reagent were established in a series of experiments.

The diazonium salt was obtained in a 5-ml. sample containing MS-222, *m*-aminoethylbenzoate, by adding 0.5 ml. of 0.2-percent sodium nitrite. The excess nitrous acid was destroyed by ammonium sulfamate. The diazonium salt was reacted with the Bratton-Marshall reagent, *N*-1-naphthylethylene-diamine dihydrochloride, to form the azo dyestuff.

The effect of diazotization time upon the concentration of azo dye formed was measured in 40 tests involving 160 reactions made with 0, 1, 3, 5, or 7.5 p.p.m. of MS-222 standards. Diazotization reactions were stopped at 3, 5, 7 to 10, and 15 to 30 minutes. The percent transmittance values were plotted for each concentration and the regression was calculated for each time interval.

The speed of azo dye formation upon addition of the coupling reagent was measured at 0, 1, 3, and 5 p.p.m. of MS-222 in standards reacted at room temperature following diazotization. The transmittance values were recorded at 30-second intervals for the first 5 minutes, 1-minute intervals for the next 10 minutes, and at 5-minute intervals up to 1 hour to measure the effect of development time.

The effect of temperature on the diazotization reaction and the resultant concentration of azo dye formed was tested at 15°, 22.5°, and 30° C. Twenty tests were conducted at the longer diazotization times at each 1-minute interval from 10 minutes through 30 minutes for each concentration of 1, 3, and 5 p.p.m. of MS-222. The mean transmittance value for each concentration and temperature was calculated for 5 measurements taken at 10-15 minutes, for 5 at 15-20 minutes, and for 10 between 20 and 30 minutes.

The maximum limit for the reaction of the diazonium ion was between 5 and 7.5 p.p.m. of MS-222 without causing precipitation on the sides of cuvettes and or optical density less than 20 percent. Thus, dilutions were designed to keep the concentration in samples in the working range of the instrumentation.

Measurements of the maximum absorbance value of the azo dye at 420 to 650

millimicrons were made with a Beckman DB spectrophotometer and a Sargent SRL recorder. Mechanical Adjustments for slit width for the maximum absorbance band were established on standard solutions of MS-222 at concentrations up to 7.5 p.p.m. with a Perkin-Elmer 139 spectrophotometer equipped with a photomultiplier.

Fish

We used rainbow trout in the tests of the residue method. The 8- to 14-inch fish were furnished by the Manchester, Iowa, National Fish Hatchery and were held in facilities of the Fish Control Laboratory. They were fed a pelleted, open-formula diet. It is important to point out that several ingredients of the diet were fortified with primary aromatic amines or vitamins such as folic acid, vitamin B₁₂, *p*-aminobenzoic acid plus protein sources such as brewer's yeast.

Tissue collection and treatment

Blood samples were drawn from specimens at specified intervals by cardiac puncture with a heparinized syringe and 18- or 20-gage needle. Within 15 minutes after the blood was taken, the samples were mixed with trichloroacetic acid (TCA) for coagulation of protein.

Each fish was immobilized by a blow on the head or pithing and the other tissues were dissected out. Skin was removed to expose the thick muscular area adjacent to the dorsal fin. A sample about 1 inch square was removed, minus bones, and placed in a tared beaker and weighed. It was homogenized and diluted 1:20 with TCA and distilled water to make up a 3-percent TCA solution.

The entire liver, excluding the gall bladder, was removed. Kidney tissue was dissected posterior to the isthmus. The tissues were weighed in tared beakers and those not processed immediately were quick-frozen with dry ice. The tissues were processed for chemical analysis in the same manner as muscle.

Analysis of residues

The concentration of aromatic amines and other masking or interfering substances were determined for blood, muscle, kidney and liver in 9 specimens of rainbow trout and expressed in terms of parts per million of MS-222. The recovery of MS-222 from each tissue was evaluated by spiking at rates of 0, 20 and 100 p.p.m. in each lot of 3 fish by using the outlined procedure.

The colorimetric reaction described by Bratton and Marshall (1939) was used, with modifications, for detection of MS-222 in fish tissues. The process was as follows:

Reagents:

1. 15-percent trichloroacetic acid: Dissolve 30 g. crystalline TCA in distilled water. Transfer to a 200-ml. volumetric flask and bring to volume with distilled water. Dilute 20 ml. to 100 to get 3-percent TCA.
2. 0.2-percent sodium nitrite: Dissolve 0.5 g. NaNO_2 in water. Transfer to a 100-ml. volumetric flask and bring to volume. Transfer 40 ml. to a second 100-ml. volumetric flask and bring to volume. Make fresh daily.
3. 0.5-percent ammonium sulfamate: Dissolve 0.5 g. in distilled water and dilute to 100 ml.
4. 0.1-percent N-1-naphthylethylenediamine dihydrochloride: Dissolve 0.5 g. in distilled water and bring to 100 ml. Transfer 20 ml. to 100-ml. volumetric flask and bring to volume. Refrigerate and make up fresh weekly.
5. 4N hydrochloric acid: Dilute 40 ml. concentrated HCl to 100 ml. Mix and titrate a 5 ml. portion with standard 1N NaOH. Adjust to exactly 4N.
6. 10-percent HCl.

Standards:

1. Stock solution: Weigh 0.1000 g. of MS-222 and dissolve in distilled water.

Quantitatively transfer to 100-ml. volumetric flask, add 20 ml. of 15-percent TCA and dilute to volume with distilled water. Stock equals 1,000 p.p.m. of MS-222 in 3-percent TCA.

2. Dilute 2.0 ml. stock to 200 ml. with 3-percent TCA. This stock contains 10 p.p.m. of MS-222.
3. Volume of 10 p.p.m. stock is diluted to 50 ml. with 3-percent TCA:

<u>Desired p.p.m.</u>	<u>ml. of 10-p.p.m. stock</u>
9.0	45.0
7.5	37.5
5.0	25.0
3.0	15.0
1.0	5.0
0.5	2.5
0.25	1.25

Procedure:

1. Sample preparation--blood and tissue.

- A. Blood: Use an anticoagulant, and draw blood with care to avoid contamination with body fluids or water. Place 0.5 ml. in 7.5 ml. of distilled water and let stand 10 minutes or until other samples are ready. Add 2 ml. of 15-percent TCA. Mix thoroughly and let set 5 minutes. Centrifuge at 2,500 RPM for 30 minutes. Filter to remove fat or other materials not spun down. Go to Part 2, Analysis.
- B. Other tissue (muscle, kidney and liver): Carefully dissect out the sample, weigh, homogenize and dilute in the following manner: One gram of tissue is homogenized and diluted to 16 ml. with distilled water in a graduated centrifuge tube. A 1:20 dilution of the sample is achieved by adding 4 ml. of 15-percent TCA. The precipitate of protein is removed by the centrifugation and filtration outlined for blood as above. The supernatant is then analyzed according to part 2, Analysis.

2. Analysis.

- A. Pipette 5 ml. of filtrate into a clean 25-ml. Erlenmeyer flask.
- B. Pipette 5 ml. of 3-percent TCA into another flask as a reagent blank.
- C. Add 0.5 ml. of 0.2-percent NaNO_2 to each flask, swirl, and let stand 15 minutes. Keep out of direct sunlight.
- D. Add 0.5 ml. of 0.5-percent ammonium sulfamate to each flask, swirl and let stand 2 to 3 minutes.
- E. Add 0.5 ml. of 0.1-percent N-1-naphthylethylenediamine dihydrochloride to each tube or flask. Swirl for 30 to 60 seconds and let stand 5 or 10 minutes. Keep out of direct sunlight.
- F. Pipette or pour into cuvettes and read in spectrophotometer at 545 millimicrons. A Model 139, Perkin and Elmer spectrophotometer with a slit width of 0.05 mm. and sensitivity of X1 was used. Sensitivity of the photomultiplier was set at 5. Obtain p.p.m. or microgram from precalibration curve or use a standard. Correct for dilution and quantity of tissue. The concentration will be in terms of free MS-222.

- G. Possible acetylated derivatives: Place 10 ml. of filtrate into a graduated centrifuge tube. Add 0.5 ml. of 4N HCl. Place in a boiling water bath for 1 hour, cool, and adjust volume to 10 ml. with water. Transfer 5 ml. to a 25-ml. Erlenmeyer flask and treat as above for free MS-222.

3. Other considerations.

- A. Bubble formation: Occasionally bubbles form in cuvettes. The cuvettes should be capped and slowly tipped. Most of the bubbles on the transparent surface should be

absorbed by the large air bubble in the cuvette. The use of larger Erlenmeyer flasks may also reduce bubble formation.

- B. Glassware cleanliness: Flasks and cuvettes which have been used in steps E and F should be rinsed, first in 10-percent HCl and then in distilled water. A colored residue may adhere to the glassware and produce errors in subsequent analyses. Several rinsings with acid are usually sufficient to remove the residue.

RESULTS

Chemistry of the colorimetric reaction

The majority of applications of the Bratton-Marshall reaction employing the N-1-naphthylethylenediamine coupling reagent have involved para-substituted compounds such as sulfonamides, procaine hydrochloride, p-aminobenzoic acid, folic acid, and chloramphenicol (Meites, 1963; Welcher, 1963; Varley, 1963). However, coupling of diazonium salts of both para- and ortho-primary aromatic amines are widely illustrated in the chemistry of azo dyestuffs (Brewster and McEwer, 1961). In our application, we are diazotizing a primary aromatic amine in the meta position to a carboxyl group. The preparation of the diazonium salt of MS-222 would probably follow the flow scheme illustrated by Natelson (1961) (fig. 1).

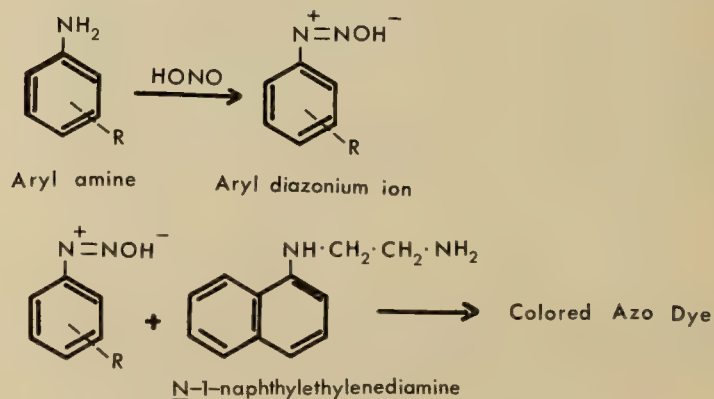


Figure 1.--Probable flow scheme of the diazotization of the ethyl ester of m-aminobenzoate (MS-222) and the coupling reaction with N-1-naphthylethylenediamine after Natelson (1961). The HCl maintains the pH at 1 to 2.

Apparently, this reaction proceeds much slower with the meta isomer than with ortho and para isomers. The suggested time for diazotization of para-substituted sulfonamides was 3 minutes with 0.1-percent sodium nitrite reagent (Bratton and Marshall, 1939). The diazotization of MS-222, however, required at least 0.2-percent sodium nitrite. A more consistent production of diazonium salt occurred at 15° to 30° C. in 15 to 30 minutes (table 1). Holbourn and Pattle (1943) observed the necessity for longer diazotization time in their work with sulfanilamide and the Bratton-Marshall reagent. They also noted the effect of light on the reaction and coupling with the reagent.

According to Marshall and Litchfield (1938) and Bratton and Marshall (1939), the ammonium sulfamate effectively destroys the excess nitrous acid. Compared to the reaction time indicated for the sulfa drugs, the diazonium salt of MS-222 appears to undergo nitroso formation at a relatively slow rate, thus providing more latitude of time prior to the termination of diazotization. Upon addition of the Bratton-Marshall reagent, N-1-naphthylethylenediamine dihydrochloride solution, the azo dyestuff formed rapidly and attained a maximum intensity within a few minutes (fig. 2). The azo dye coupling reaction proceeds immediately to form a wine-red colored azo dyestuff with a maximum absorbance in the spectral range of 545 millimicrons (fig. 3). This absorbance curve is very similar to that illustrated for sulfa drugs by Natelson (1961). The absorbance values or percent transmittance were plotted

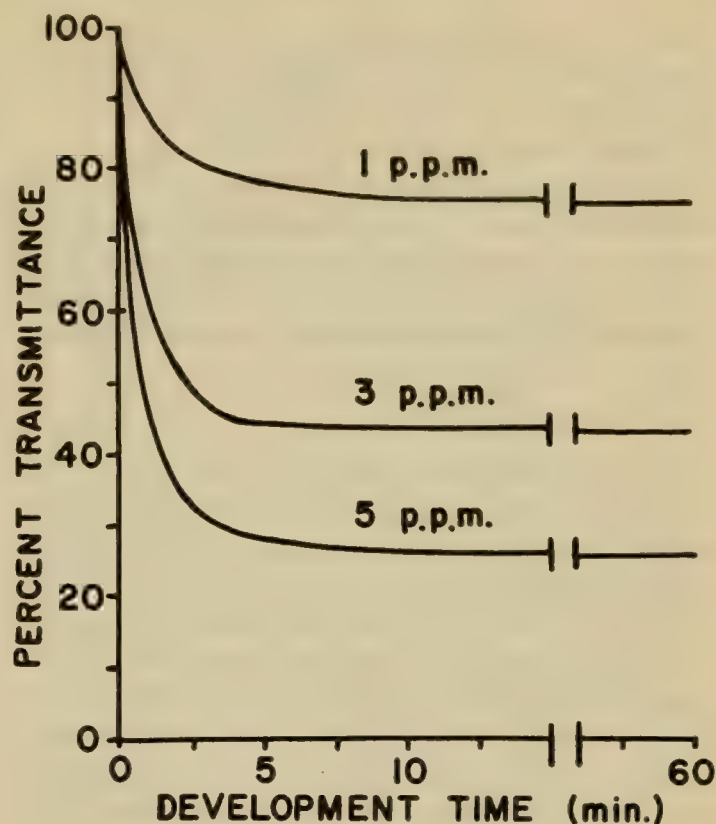


Figure 2.--Speed of development of the azo dyestuff in the coupling reaction of N-1-naphthylethylenediamine with three concentrations of diazotized MS-222.

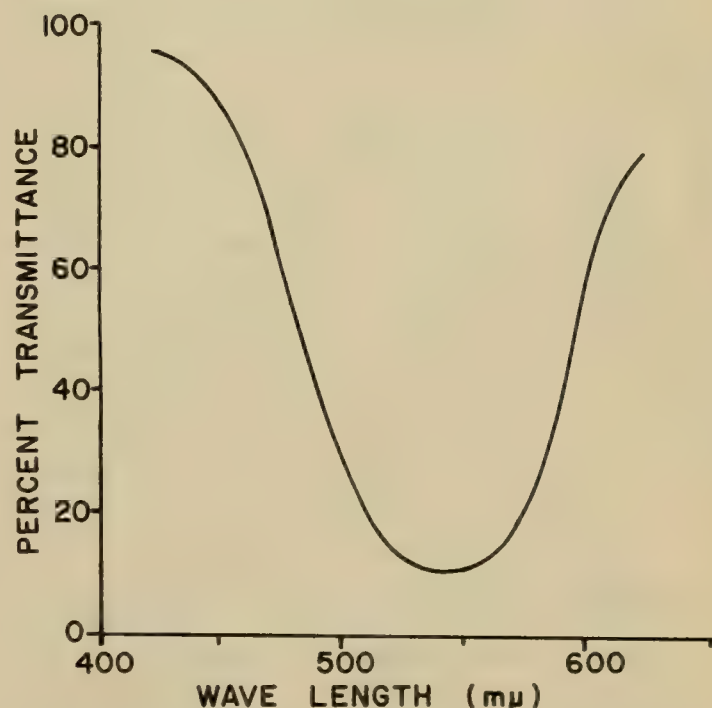


Figure 3.--Absorption spectrum for the azo dyestuff developed in the reaction of diazotized MS-222 and N-1-naphthylethylenediamine.

TABLE 1.--Effect of diazotization times at three temperatures on the percentage of transmittance values observed for MS-222 in the Bratton-Marshall reaction

Diazotization time and temperature	Percentage of transmittance at--					
	1 p.p.m.		3 p.p.m.		5 p.p.m.	
	Mean	Range	Mean	Range	Mean	Range
10-15 minutes:						
15.0° C.....	78.3	77.8-81.5	48.4	46.9-50.3	29.7	29.5-30.6
22.5° C.....	77.8	76.5-80.4	47.7	45.0-48.4	28.7	27.5-30.2
30.0° C.....	77.0	76.5-78.8	46.9	45.0-47.6	28.2	27.4-29.3
15-20 minutes:						
15.0° C.....	77.2	76.8-79.1	47.0	46.3-50.0	28.1	27.6-29.5
22.5° C.....	76.8	76.8-77.6	46.6	45.5-47.0	27.5	26.5-28.9
30.0° C.....	76.3	75.7-77.0	46.0	45.0-46.7	27.0	26.3-27.9
20-30 minutes:						
15.0° C.....	76.1	75.8-76.5	44.6	43.9-45.0	26.2	25.8-26.6
22.5° C.....	76.6	75.0-76.1	44.0	43.3-44.4	25.8	25.2-26.2
30.0° C.....	75.3	74.9-75.8	43.5	43.0-44.0	25.3	24.7-25.8

on the logarithmic scale versus the concentration in p.p.m. as MS-222 on an arithmetic scale with 1-cycle log-arithmetic paper (fig. 4). A straight line relation exists between means of points established at 1, 3, and 5 p.p.m., and it adheres to Beer's law over a wide range of diazotization times. Greater sensitivity was obtained at 15 to 30 minutes, and the values did not vary as greatly as those at shorter diazotization periods.

The accuracy of the determination is greatest in the middle section of the curve. Hawk et al. (1954) mention that higher concentrations produce colors too deep for precise measurement. The dyestuff appears to precipitate at the liquid-air interface at the higher concentration of MS-222, and it adheres to the sides of glassware. It is exceedingly important that cuvettes be rinsed with 10-percent HCl prior to introducing the developed sample. We also found it expedient to rinse the cuvettes at least once with the

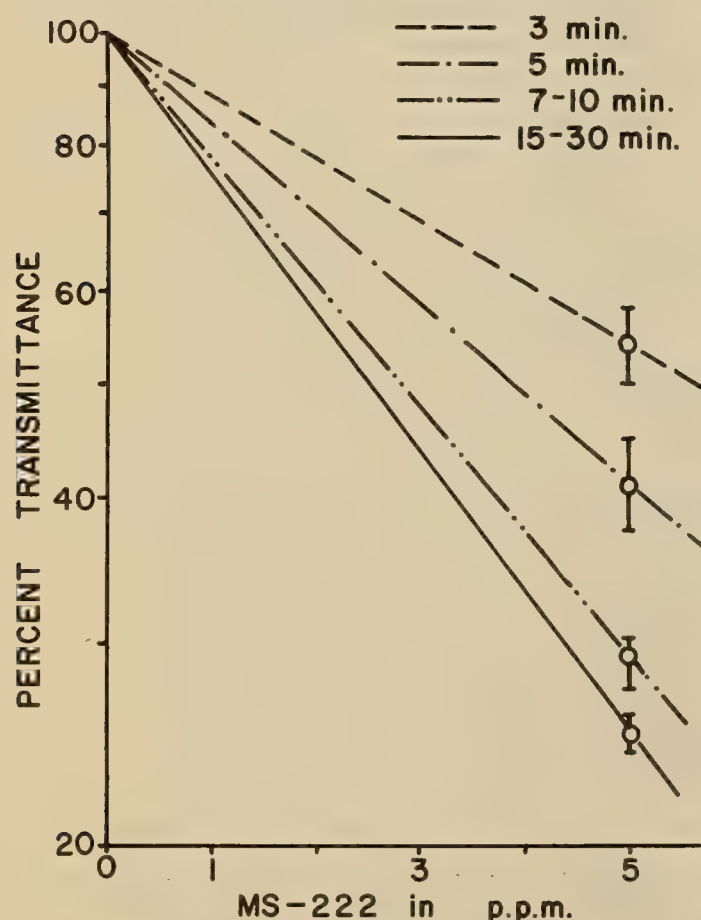


Figure 4.--Transmittance curves of MS-222 showing the effects of different diazotization times on the intensity of color development at 545 millimicrons and the range of values observed at 5 p.p.m.

developed sample solution to avoid a negative dilution error. Contaminants and/or gas bubbles on the sides of cuvettes in the light path may produce serious quantitative errors at high transmittance. Particular care must be taken to remove detergent film from glassware by a thorough rinsing with dilute HCl and deionized water. Detergents cause yellow or green-brown turbidity in extreme cases of contamination. This turbidity is distinguishable from the nitroso formation of azoxy compounds of MS-222 and natural aromatic amines which are usually reddish-brown in color.

Measuring MS-222 in fish

Since the Bratton-Marshall color reaction is specific to aromatic amines, the quantitation of MS-222 has to be differentiated from natural occurring amines such as *p*-amino-benzoic acid (Hawk et al., 1954). Thus, we determined background levels of amines and other masking substances in blood, muscle, kidney, and liver (table 2). Further, the amounts of recoverable MS-222 were determined by spiking samples of two concentrations (table 3). This determined its effectiveness in measuring different concentrations in the various tissues over and above background interferences. Recoveries ranged from 89 to 112 percent for all samples.

TABLE 2.--Concentrations of background at 545 millimicrons in rainbow trout

Tissue	Number of fish	Background		Standard error	95-percent confidence interval
		Mean (p.p.m.)	Range (p.p.m.)		
Blood.....	9	0.91	0.0- 2.0	0.19	0.46- 1.36
Muscle.....	9	0.87	0.0- 2.0	0.29	0.21- 1.53
Kidney.....	9	5.42	2.8- 9.0	0.58	4.08- 6.76
Liver.....	9	18.13	11.6-20.0	0.96	15.92-20.34

TABLE 3.--Recovery of MS-222 spiked into various tissues of rainbow trout

Tissue	Number of fish	Concentration of spikes (p.p.m.)	MS-222 in tissue		
			Mean (p.p.m.)	Range (p.p.m.)	Percent recovery
Blood.....	3	20	22.47	21.6- 23.8	112.3
	3	100	99.47	96.6-101.0	99.5
Muscle.....	3	20	21.00	20.6- 21.4	105.0
	3	100	101.87	94.2-106.2	101.9
Kidney.....	3	20	18.27	17.8- 18.6	91.3
	3	100	88.93	86.2- 91.6	88.9
Liver.....	3	20	20.40	19.2- 21.4	101.7
	3	100	103.30	95.2-109.6	103.3

Mooney and Pasarela (1964) refined the cleanup of samples to reduce contamination by procaine penicillin and other interfering substances in the background. They employed a Dow-50W-X2 (H^+) ion exchange resin to extract sulfonamides selectively in acid phase and to elute them in alkaline phase. This method was not applicable to the cleanup of fish tissues because we could not elute MS-222 from the column.

Liver gave the most interference by producing a true red, azo dye color reaction, and the levels were quite consistent with a small deviation and narrow confidence limits. Kidney samples were characterized by a yellow color. This pigment introduced a negative interference with the azo dye reaction, but gave a consistent recovery of spiked MS-222. Blood and muscle gave relatively little color reaction or interference. The volumes of blood samples, however, were small, and contributed to positive dilution errors. The errors were particularly significant in the recovery of lower concentrations of MS-222. In general, the overall accuracy of the method is governed by the techniques of extraction and dilution.

CONCLUSIONS

MS-222 can be detected in fish tissues by means of a modified Bratton-Marshall method. Interfering substances were more prevalent in liver and kidney than in blood and muscle. The recovery of MS-222 from spiked samples ranged from 89 to 112 percent. The method was more accurate for measuring MS-222 in blood and muscle than in kidney and liver.

SUMMARY

Use of MS-222 as a fish anesthetic necessitated an investigation into the residues in fish following exposure. Fortunately, the toxicity of MS-222 to mammals was found to be quite low with an acute oral LD_{50} of approximately 5-10 g./kg. to laboratory rats. Thus, an analytical procedure which would

define concentrations in p.p.m. was considered adequate.

MS-222 has the chemical structure of a primary aromatic amine and it reacts colorimetrically with the Bratton-Marshall reagent. We modified the method commonly used for determination of sulfa drugs.

MS-222 forms a diazonium salt much more slowly than the para-substituted aromatic amines and thus required a higher concentration of nitrous acid and longer time for development. The azo dye coupling reaction proceeds almost immediately with N-1-naphthylethylenediamine dihydrochloride to form a wine-red dye complex with a maximum absorbance in the spectral range of 545 millimicrons. The regression of absorbance values was almost linear with a slope of 1.0, and it bore evidence of adherence to Beer's law.

The fish tissues were extracted in distilled water, and the protein was precipitated in a 3-percent TCA solution. The solution was centrifuged and then filtered to remove the lighter fatty fraction. A 1-to-20 dilution was necessary to adjust the concentration within the limits of the transmission curve (0 to 7.5 p.p.m.).

We obtained excellent recoveries of MS-222 from all spiked samples of fish tissue. They ranged from 89 to 112 percent which excluded the aromatic amines of natural origin. The background of primary aromatic amines varied with each tissue and between individual specimens. The backgrounds were higher in liver and kidney, and residues of MS-222 were more difficult to differentiate. In liver, it was due, presumably, to the diazotized amines such as vitamin B_{12} , p-aminobenzoic acid, folic acid, and the many proteins in fortified diets for trout. A yellow pigment persisted in extracts from kidneys which introduced a negative interference with the azo dye reaction. The modified Bratton and Marshall method was, however, particularly effective in determining residues of MS-222 in muscle and blood of trout.

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15. Residues of MS-222 in Four Salmonids Following Anesthesia

By Charles R. Walker and Richard A. Schoettger



United States Department of the Interior, Stewart L. Udall, *Secretary*
Stanley A. Cain, *Assistant Secretary for Fish and Wildlife and Parks*
Fish and Wildlife Service, Clarence F. Pautzke, *Commissioner*
Bureau of Sport Fisheries and Wildlife, John S. Gottschalk, *Director*
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RESIDUES OF MS-222 IN FOUR SALMONIDS FOLLOWING ANESTHESIA

By Charles R. Walker, Chemist and Richard A. Schoettger, Fishery Biologist
Bureau of Sport Fisheries and Wildlife
La Crosse, Wisconsin

Abstract.--Residues of MS-222 (tricaine methanesulfonate) in the blood, muscle, liver, and kidney of rainbow trout and in the muscle of brown trout, brook trout, and lake trout were measured by a modified Bratton-Marshall colorimetric method. Temperatures were 7°, 12°, and 17° C. in waters with total hardnesses of 10 to 180 p.p.m. The residues were easily detected and measured in blood and muscle, but they were masked in liver and kidney by background substances. The anesthetic dissipated rapidly in the muscle of the four species within 1 to 6 hours, and the residues approached the background levels of controls within 9 to 24 hours after withdrawal from exposure. The differences in residue levels in the muscle at 0-hour withdrawal between species, at three temperatures, and in soft and hard water largely disappeared within 24 hours.

MS-222 (tricaine methanesulfonate) has gained wide acceptance as a fish anesthetic and transportation sedative. There has been no investigation, however, on its residues in fish following treatment. This information is especially important in situations where fish exposed to anesthetic or sedating solutions are eaten soon after by people.

An extensive review of the literature on the many uses of MS-222 was made by Schoettger (1966). Schoettger and Julin (1966) defined the criteria for desired stages of anesthesia, and the efficacy of MS-222 at various concentrations and times of exposure to rainbow trout, brown trout, brook trout, and lake trout. The analyses of residues of the anesthetic in these species were performed concurrently with the investigations of efficacy.

We selected the extremes of concentration and depth of anesthesia to ascertain the existence and persistence of residues in the four trouts. The major emphasis was placed on the determination of residues in muscle

since it is more important than other tissues in human consumption. The rainbow trout, the species more widely propagated for sport and food in the United States, was used for evaluation of residues in other tissues: blood, liver and kidney. We also concentrated observations at 12° and to lesser extent at 7° and 17° C. This range of temperature is considered to be the most suitable for trout production (Davis, 1956).

METHODS AND MATERIALS

Fish

The rainbow trout, brown trout, brook trout, and lake trout ranged from 7 to 14 inches long, and were obtained from State fish hatcheries at Lanesboro, Minn. and St. Croix, Wis., and from national fish hatcheries at Jordan River, Mich., Lake Mills, Wis. and Manchester, Ia. They were held in well water and facilities described by Lennon and Walker (1964) and taken off feed for

at least 24 hours before treatment with MS-222.

Anesthetization

Schoettger and Julin (1966) established the efficacy of MS-222 as an anesthetic for several species of trout at various temperatures and water qualities. Concurrent with these studies, we sampled tissues from the fish which were anesthetized at 7, 12 and 17° C. The concentrations of anesthetic solutions ranged from 80 to 135 p.p.m., and the mean time to reach the desired stage of anesthesia ranged from 4 to 12 minutes depending on temperature and species. After specimens reached deep anesthesia, they were withdrawn from the drug solution and placed in fresh water for recovery. This action marked the beginning of withdrawal time.

Collection and analyses of tissues

The methods used for collecting and analyzing the blood, muscle, liver, and kidney of trout were described by Walker and Schoettger (1966).

RESULTS

Preliminary analyses

Initially we sought to detect and measure residues of MS-222 in blood, muscle, liver, and kidney of rainbow trout at intervals of 0, 5, 10, 20, and 30 minutes after withdrawal from 9 minutes of exposure to 100 p.p.m. of drug at 12° C. The 12- to 14-inch fish were in advanced loss of reflex and some had reached medullary collapse (table 1). Residues occurred in all tissues and the higher concentrations were found in blood, liver, and kidney at 0-minute withdrawal. The higher concentration of residue in muscle was detected at the 10-minute withdrawal. The results were variable. It was apparent that withdrawal time should be extended beyond 30 minutes, more samples should be used, and all specimens should be in the same stage of anesthesia.

TABLE 1.--Residues of MS-222 including background amines recovered from adult rainbow trout following withdrawal from anesthesia

Fish sample	Interval after withdrawal (minutes)	Residues in p.p.m.			
		Blood	Muscle	Liver	Kidney
No. 1.....	0	61.6	7.3	49.5	91.5
No. 2.....	0	--	6.7	53.7	52.3
No. 3.....	5	17.6	1.3	21.3	29.2
No. 4.....	5	20.2	9.8	14.6	27.8
No. 5.....	10	13.0	18.9	25.4	23.9
No. 6.....	10	9.1	1.1	8.3	9.4
No. 7.....	15	6.9	1.1	37.4	9.6
No. 8.....	15	9.5	7.3	11.5	10.6
No. 9.....	20	7.1	9.2	41.3	14.9
No. 10.....	20	6.3	4.2	23.6	5.9
No. 11.....	30	5.9	4.0	11.5	8.7
No. 12.....	30	5.1	0.7	11.5	2.3

Another group of 15 rainbow trout, 12 to 14 inches long, was anesthetized in 100 p.p.m. of MS-222 at 12° C. to a state of medullary collapse. Tissues were sampled for residues at 0, 1, and 2 hours following withdrawal (table 2). The concentrations of the drug were highest in the blood of fish at 0-hour withdrawal. They dissipated rapidly as shown in a regression (fig. 1).

A similar pattern was noted in the muscle, although the initial concentration of residue was somewhat lower (table 2 and fig. 2). The regressions indicate that the amounts of MS-222 in blood and muscle approach background quantities of aromatic amines after 2 hours of withdrawal from the anesthetic solution. The 95-percent confidence intervals overlap at the 2-hour withdrawal.

The livers of untreated fish had a wide range in concentration of background amines, and it was difficult to distinguish between them and actual residues of MS-222 (table 2). The apparent concentration of the drug in liver was highest at the 1-hour reading and fell off in 2 hours to values only slightly above controls. An extrapolation of the regression indicates that residues of the drug persist beyond 5 hours (fig. 1).

The residues of MS-222 in kidney were somewhat more persistent than those in muscle but also declined during the 2-hour period following withdrawal (table 2). A projection of the regression makes an interception very close to the 2-hour reading (fig. 1). The broad confidence intervals at the 1- and 2-hour withdrawals overlap

TABLE 2.--Residues of MS-222 including background amines recovered from adult rainbow trout following withdrawal from anesthesia

Tissue and withdrawal interval	Number of fish	Mean size		Residues in p.p.m.		Standard error	95-percent confidence interval
		Length (inches)	Weight (grams)	Mean	Range		
Blood:							
Control.....	5	13.4	425.0	2.60	1.2- 4.2	0.56	1.05- 4.14
0 hour.....	5	13.2	412.4	69.40	63.4-78.4	3.11	60.77-78.03
1 hour.....	5	12.6	319.0	15.88	4.8-28.2	0.56	14.34-17.42
2 hours.....	5	12.8	348.0	5.96	4.0-10.0	1.09	2.94- 8.98
Muscle:							
Control.....	5	13.4	425.0	1.92	0.8- 3.6	0.50	0.53- 3.31
0 hour.....	5	13.2	412.4	7.52	2.2-11.4	1.61	3.06-11.98
1 hour.....	5	12.6	319.0	4.28	2.6- 7.6	0.63	2.53- 6.03
2 hours.....	5	12.8	348.0	3.52	2.6- 4.6	0.32	2.63- 4.41
Liver:							
Control.....	5	13.4	425.0	38.44	31.0-50.2	3.36	29.13-47.75
0 hour.....	5	13.2	412.4	46.00	34.6-71.0	7.02	26.51-65.49
1 hour.....	5	12.6	319.0	52.68	41.0-67.4	4.62	39.87-65.49
2 hours.....	5	12.8	348.0	43.00	29.4-63.0	5.99	26.37-59.63
Kidney:							
Control.....	5	13.4	425.0	15.32	11.8-16.2	0.88	12.88-17.76
0 hour.....	4	13.1	408.5	55.85	47.8-70.0	5.10	39.61-72.09
1 hour.....	4	12.6	315.8	24.20	16.0-46.4	7.41	0.64-47.76
2 hours.....	5	12.8	348.0	17.20	14.2-19.2	0.82	4.92-19.48

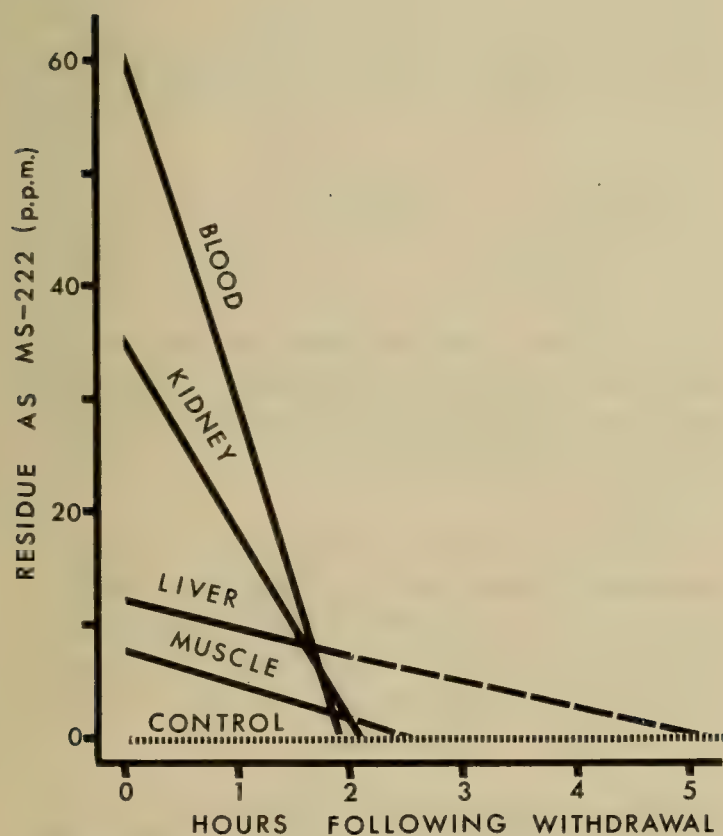


Figure 1.--Regressions of the residues of MS-222 in blood, muscle, liver, and kidney of rainbow trout on withdrawal time. The residues are expressed as concentration above the mean background value for control fish.

considerably. We also found that the high levels of background in untreated controls differed slightly between these time intervals.

The uptake of MS-222 was higher in individuals which were anesthetized in soft water

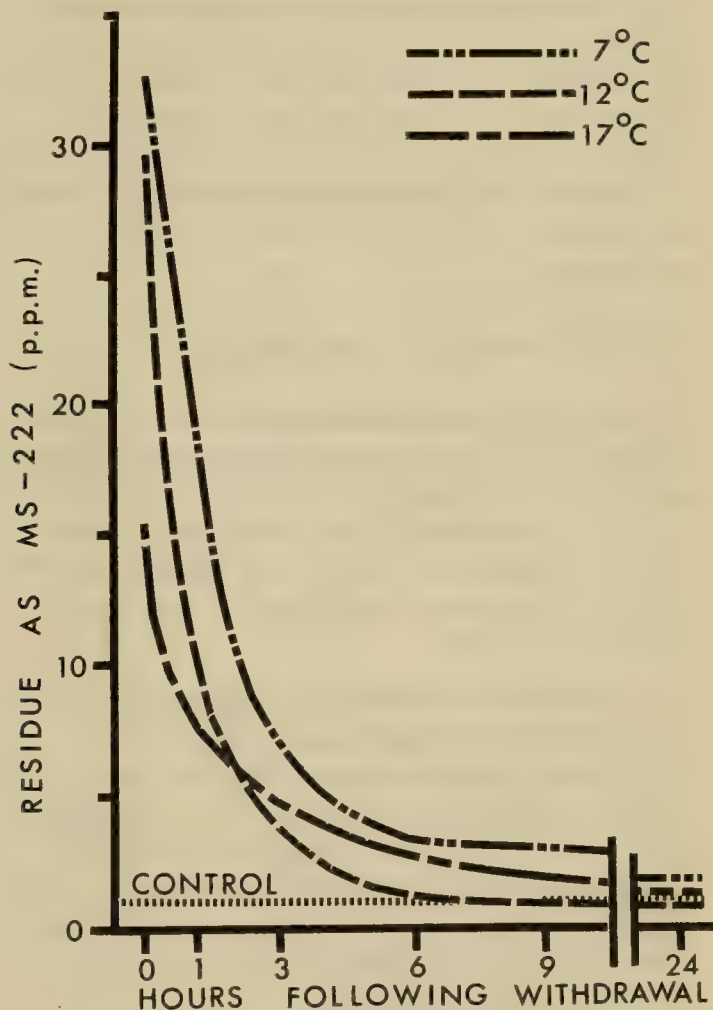


Figure 2.--Regression of the residues of MS-222 in muscle of four species of trout on withdrawal time following deep anesthesia at three temperatures. The lines were drawn through mean values for residues at each withdrawal.

than those treated in hard water (table 3). The greater uptake of drug in soft water appeared to be related to longer exposures. The times

TABLE 3.--Residues of MS-222 including background in muscle of rainbow trout following deep anesthesia in hard and soft water at 12° C.

Hardness and withdrawal interval	Number of fish	Mean size		Residues in p.p.m.		Standard error	95-percent confidence interval
		Length (inches)	Weight (grams)	Mean	Range		
Hard water (180 p.p.m.):							
0 hour.....	3	11.5	233.3	9.60	3.4-16.0	2.10	0.99-18.21
24 hours.....	3	10.6	188.3	2.37	2.1- 2.6	0.09	2.01- 2.73
Soft water (10 p.p.m.):							
0 hour.....	3	11.5	233.3	30.73	27.6-35.6	1.43	24.39-36.57
24 hours.....	3	11.1	216.3	2.47	2.2- 2.8	0.10	2.03- 2.90

for anesthetization of fish in soft water were approximately double those in hard water, but recoveries were more rapid (Schoettger and Julin, 1966). The concentrations of acetylated MS-222 in fish anesthetized in soft and hard water were approximately the same. However, they amounted to 20 percent of the total aromatic amines which were measured in fish treated in soft water as compared with 40 percent for fish in hard water at the 0-hour withdrawal. We were unable to show any differences in concentrations of free MS-222 at 24 hours, and approximately 30 to 35 percent of the total aromatic amines were acetylated.

Intensive analyses on four species

Rainbow trout.--Seventy-five 8- to 12-inch rainbow trout were anesthetized to medullary collapse in 100 p.p.m. of MS-222 at 7°, 12°, and 17° C. (table 4). The induction of anesthesia ranged from 6.8 to 11.4 minutes at 7°, 8.0 to 11.9 minutes at 12°, and 5.5 to 6.9 minutes at 17° C.

The residues including background in blood at 0-hour withdrawal ranged from 39.8 to 65.8 p.p.m., with the least variation at the higher temperature (table 4). They decreased rapidly at 7° and 12° compared with 17° C. The levels of background in these tests were 0.8 to 1.0 p.p.m. (table 5). Thus, the residues of MS-222 approach the background levels after 24 hours withdrawal.

The highest concentrations of residues of MS-222 including background in muscle occurred at 0-hour withdrawal at all water temperatures (table 6). They varied greatly between individual fish, thereby causing large standard errors and making the calculation of 95-percent confidence intervals impractical. The standard errors decreased as the

TABLE 4.--Residues of MS-222 including background in blood of rainbow trout following deep anesthesia at selected time intervals and water temperatures

Temperature and withdrawal interval	Number of fish	Mean size		Residues in p.p.m.		Standard error
		Length (inches)	Weight (gram)	Mean	Range	
7° C.:						
0 hour.....	2	10.00	154.0	52.8	39.8-65.8	13.00
6 hours.....	2	9.70	131.5	2.5	2.0- 3.0	0.50
9 hours.....	2	9.95	141.0	1.6	1.4- 1.8	0.20
24 hours.....	1	10.00	154.0	1.0	--	--
12° C.:						
0 hour.....	2	9.85	141.5	60.6	57.2-64.0	3.40
1 hour.....	2	10.00	150.5	6.2	5.6- 6.8	0.60
3 hours.....	2	9.80	150.0	2.1	2.0- 2.2	0.10
6 hours.....	1	10.10	155.0	2.6	--	--
9 hours.....	2	9.25	118.0	1.9	1.8- 2.0	0.10
17° C.:						
0 hour.....	2	10.55	187.0	45.8	43.8-47.8	2.00
6 hours.....	2	10.15	159.5	5.1	4.8- 5.4	0.30
9 hours.....	2	10.25	166.5	4.6	4.6- 4.6	0.00
24 hours.....	2	10.40	168.0	1.4	1.0- 1.8	0.40

time of withdrawal increased. Furthermore, the residues of 9.4 to 72.0 p.p.m. at 0-hour withdrawal declined rapidly to a range of 0.8 to 3.8 p.p.m. at 6 hours.

The mean residues in muscle at the 9-hour withdrawal were equal to or less than background values, and the confidence intervals were similar (table 5). It appears that no significant residues of MS-222 remain in muscle at 9 hours after withdrawal.

The residues of MS-222 in livers exceeded the mean background by 17 to 59 p.p.m. at 0-hour withdrawal (table 7). Thereafter, the residues were masked by the high and variable levels of background amines (table 5).

The residues in kidneys at 0-hour withdrawal exceeded the mean background by 21 to 90 p.p.m. (table 8). At 7° and 17°, the mean residues at 9- to 24-hour withdrawals were below the means of background in untreated controls (tables 5 and 7). At 12°, the mean residues beyond 3 hours of withdrawal fell within the range of background values.

TABLE 5.--Background residues of aromatic amines in tissues of fish which were used as untreated controls in the study of MS-222

Species and tissue	Number of fish	Mean size		Residues in p.p.m.		Standard error	95-percent confidence interval
		Length (inches)	Weight (grams)	Mean	Range		
Rainbow trout:							
Blood.....	2	9.22	110.80	0.90	0.8- 1.0	0.22	--
Muscle.....	5	9.22	110.80	1.44	0.8- 2.2	0.26	0.72- 2.16
Liver.....	5	9.22	110.80	13.72	11.6-16.0	0.72	9.60-18.00
Kidney.....	5	9.22	110.80	7.12	2.6-10.4	1.36	4.34-10.90
Brown trout: Muscle.....	3	7.60	71.67	0.40	0.2- 0.6	0.12	0.00- 0.92
Brook trout: Muscle.....	3	9.53	137.33	0.53	0.4- 0.6	0.07	0.23- 0.83
Lake trout: Muscle.....	3	7.60	45.67	0.40	0.2- 0.6	0.11	0.00- 0.87

TABLE 6.--Residues of MS-222 including background in muscle of rainbow trout following deep anesthesia at selected water temperatures

Temperature and withdrawal interval	Number of fish	Mean size		Residues in p.p.m.		Standard error	95-percent confidence interval
		Length (inches)	Weight (grams)	Mean	Range		
7° C.:							
0 hour.....	5	9.50	127.2	41.92	21.4-72.0	7.13	--
6 hours.....	5	9.26	119.6	1.48	1.0- 2.2	0.21	0.58- 2.38
9 hours.....	5	9.82	135.8	1.00	0.8- 1.2	0.06	0.74- 1.26
24 hours.....	5	9.78	141.2	0.80	0.6- 1.0	0.06	0.54- 1.06
12° C.:							
0 hour.....	5	8.14	86.2	26.40	10.2-50.8	7.20	--
1 hour.....	5	9.64	129.4	9.72	7.4-17.2	1.92	0.46-17.98
3 hours.....	5	8.64	102.6	2.64	1.6- 4.4	0.48	0.57- 4.71
6 hours.....	5	8.94	116.0	1.04	0.8- 1.4	0.10	0.61- 1.47
9 hours.....	5	8.32	87.6	1.00	0.8- 1.4	0.14	0.60- 1.40
24 hours.....	5	8.76	95.8	1.44	1.0- 2.0	0.20	0.58- 2.30
17° C.:							
0 hour.....	5	10.22	167.4	18.04	9.4-23.8	2.58	--
6 hours.....	5	9.62	133.4	3.44	2.8- 3.8	0.17	2.71- 4.17
9 hours.....	5	9.82	143.8	1.52	0.8- 2.8	0.34	0.06- 2.98
24 hours.....	5	10.02	149.8	1.24	1.0- 1.6	0.11	0.77- 1.71

TABLE 7.--Residues of MS-222 including background (grams) in livers of rainbow trout following deep anesthesia at selected time intervals and water temperatures

Temperature and withdrawal interval	Number of fish	Mean size		Residues in p.p.m.		Standard error
		Length (inches)	Weight (grams)	Mean	Range	
7° C.:						
0 hour.....	2	10.00	154.0	59.6	46.2-73.0	13.39
6 hours.....	2	9.70	131.5	17.5	15.8-18.2	1.20
9 hours.....	2	9.95	141.0	20.0	18.2-21.8	1.80
24 hours.....	2	10.00	146.5	15.0	14.8-15.2	0.06
12° C.:						
0 hour.....	2	9.85	141.5	60.6	57.2-64.0	3.40
1 hour.....	2	10.00	150.5	19.5	18.4-20.6	1.10
3 hours.....	2	9.80	150.0	17.9	16.0-19.8	1.90
6 hours.....	2	9.50	132.0	11.1	11.0-11.2	0.06
9 hours.....	2	9.25	118.0	13.4	12.0-14.8	1.39
17° C.:						
0 hour.....	2	10.55	187.0	49.3	31.0-67.6	18.30
6 hours.....	2	10.15	159.5	20.5	19.6-21.4	0.90
9 hours.....	2	10.25	166.5	17.5	17.0-18.0	0.50
24 hours.....	2	10.40	168.0	13.7	13.0-14.4	0.70

TABLE 8.--Residues of MS-222 including background (grams) in kidneys of rainbow trout following deep anesthesia at selected time intervals and water temperatures

Temperature and withdrawal interval	Number of fish	Mean size		Residues in p.p.m.		Standard error
		Length (inches)	Weight (grams)	Mean	Range	
7° C.:						
0 hour.....	2	10.00	154.0	84.2	71.4-97.0	12.79
6 hours.....	2	9.70	131.5	9.4	9.2- 9.6	0.20
9 hours.....	2	9.95	141.0	6.6	6.0- 7.2	0.60
24 hours.....	2	10.00	146.5	6.4	6.0- 6.8	0.40
12° C.:						
0 hour.....	2	9.85	141.5	38.0	28.2-47.8	9.80
1 hour.....	2	10.00	150.5	15.5	11.6-19.4	3.90
3 hours.....	2	9.80	150.0	10.8	7.8-13.8	3.00
6 hours.....	2	9.50	132.0	8.9	8.4- 9.4	0.50
9 hours.....	2	9.25	118.0	9.1	7.0-11.2	2.10
17° C.:						
0 hour.....	2	10.55	187.0	62.8	58.8-66.8	4.00
6 hours.....	1	10.30	170.0	11.2	- -	-
9 hours.....	2	10.25	166.5	8.0	5.8-10.2	2.20
24 hours.....	2	10.40	168.0	5.4	5.2- 5.6	0.20

The reliability of the modified Bratton-Marshall colorimetric reaction for detection and measurement of MS-222 in liver and kidney is compromised by the high and variable concentrations of background amines. Therefore, residues in these tissues were measurable only at 0-hour withdrawal. The detection of residues in these organs is of little importance other than to indicate the ability of fish to deactivate or metabolize the anesthetic. The liver and kidney are rarely consumed by people, and they comprise only a small fraction of the total weight of a fish.

Brown trout.--Groups of 6- to 9-inch brown trout were brought to medullary collapse by 80 p.p.m. of MS-222 at 17° within 4.6 to 7.0 minutes. Because the species is less sensitive to the drug at 7° and 12°, 100-p.p.m. solution was used to achieve medullary collapse within 9.5 to 12.1 minutes at 7° and within 7.9 to 11.0 minutes at 12°.

The residues of MS-222 in brown trout were measured only in muscle (table 9). The concentrations, including background, ranged from 9.8 to 60.8 p.p.m. at 0-hour withdrawal and declined 80 to 99 percent within 6 hours. They were 0.4 to 5.4 p.p.m. at 6 hours and 0.6 to 1.4 p.p.m. at 24 hours. Also, they were least variable at 17° and persisted longer at 7°. After 24 hours, the mean residues of MS-222 were only a fraction of a part per million over background.

Brook trout.--Brook trout 7 to 10 inches long were anesthetized to medullary collapse. The concentrations of drug were 100 p.p.m. at 17°, 110 p.p.m. at 12°, and 120 p.p.m. at 7°. Anesthesia was induced within 4.2 to 5.4 minutes at 17°, 6.5 to 8.7 minutes at 12°, and 8.8 to 11.4 minutes at 7°.

The residues, including background, in muscle ranged from 6.0 to 34.8 p.p.m. at 0-hour withdrawal at the three temperatures (table 10). This range was lower than in any of the other trout, and it is noteworthy because the brook trout required higher concentrations of the drug for anesthesia. Within 9 to 24 hours after withdrawal, the residues including background were 0.6 to 2.8 p.p.m. At 24 hours, the mean residues of MS-222 exceeded the backgrounds by 1.1 p.p.m. at 17°, 0.3 p.p.m. at 12°, and 1.4 p.p.m. at 7°, but their 95-percent confidence intervals overlapped.

Lake trout.--Lake trout 6 to 8 inches long were brought to medullary collapse by 100 p.p.m. of MS-222 at 17°, 110 p.p.m. at 12°, and 135 p.p.m. at 7° within 3.9 to 6.5 minutes overall. The highest concentrations of residues, including background, were measured in muscle from fish at 7°. These fish had longer exposures to higher concentrations of drug than the others.

The mean values for residues of anesthetic at 6-, 9-, and 24-hour withdrawals at the

TABLE 9.--Residues of MS-222 including background in muscle of brown trout following deep anesthesia at selected time intervals and water temperatures

Temperature and withdrawal interval	Number of fish	Mean size		Residues in p.p.m.		Standard error	95-percent confidence interval
		Length (inches)	Weight (grams)	Mean	Range		
7° C.:							
0 hour.....	3	8.03	83.3	27.53	9.8-43.4	9.75	--
6 hours.....	3	8.17	88.0	5.07	4.8- 5.4	0.18	4.30-5.84
9 hours.....	3	8.03	89.0	4.67	4.4- 4.6	0.18	3.90-5.44
24 hours.....	3	7.93	83.3	1.13	1.0- 1.4	0.23	0.14-2.12
12° C.:							
0 hour.....	3	7.40	55.0	44.20	20.8-60.8	12.05	--
1 hour.....	3	7.77	71.3	5.67	4.6- 7.4	0.88	1.88-9.46
3 hours.....	3	7.83	73.7	1.67	1.4- 2.0	0.18	0.90-2.44
6 hours.....	3	7.17	55.7	0.60	0.4- 0.8	0.11	0.13-1.37
9 hours.....	3	7.60	63.0	1.03	0.7- 1.4	0.20	0.14-1.89
24 hours.....	3	7.80	76.7	0.80	0.6- 1.0	0.11	0.33-1.27
17° C.:							
0 hour.....	3	7.80	73.3	12.93	12.2-23.2	0.37	--
6 hours.....	3	7.37	59.7	2.20	2.0- 2.4	0.11	1.73-2.67
9 hours.....	3	7.90	69.7	1.80	1.6- 2.0	0.11	1.33-2.27
24 hours.....	3	6.93	50.0	1.40	1.4- 1.4	0.00	1.40-1.40

TABLE 10.--Residues of MS-222 including background in muscle of brook trout following deep anesthesia at selected time intervals and water temperatures

Temperature and withdrawal interval	Number of fish	Mean size		Residues in p.p.m.		Standard error	95-percent confidence interval
		Length (inches)	Weight (grams)	Mean	Range		
7° C.:							
0 hour.....	3	8.00	138.3	16.67	6.0-22.6	5.34	--
6 hours.....	3	8.83	123.0	3.53	3.2- 3.8	0.18	2.76-4.30
9 hours.....	3	9.00	118.3	2.60	2.4- 2.8	0.11	2.13-3.07
24 hours.....	3	8.43	94.7	1.93	1.8- 2.2	0.23	0.94-2.92
12° C.:							
0 hour.....	3	9.23	118.0	28.07	17.8-34.8	5.22	--
1 hour.....	3	8.77	110.3	3.73	3.2- 4.6	0.44	1.84-5.62
3 hours.....	3	9.63	148.3	1.37	0.7- 1.8	0.34	0.00-2.83
6 hours.....	3	8.20	81.7	0.83	0.8- 0.9	0.03	0.70-0.96
9 hours.....	3	8.70	106.7	1.03	0.9- 1.2	0.10	0.60-1.46
24 hours.....	3	8.60	90.7	0.80	0.6- 1.0	0.11	0.33-1.27
17° C.:							
0 hour.....	3	8.93	112.0	15.47	13.0-19.0	1.86	--
6 hours.....	3	9.73	143.0	3.33	3.0- 3.6	0.18	2.56-4.10
9 hours.....	3	8.33	110.0	2.33	2.2- 2.4	0.07	2.03-2.63
24 hours.....	3	8.83	102.0	1.67	1.2- 2.0	0.24	0.64-2.70

TABLE 11.--Residues of MS-222 including background in muscle of lake trout following deep anesthesia at selected time intervals and water temperatures

Temperature and withdrawal interval	Number of fish	Mean size		Residues in p.p.m.		Standard error	95-percent confidence interval
		Length (inches)	Weight (grams)	Mean	Range		
7° C.:							
0 hour.....	3	7.43	46.3	32.00	20.0-55.6	11.82	--
6 hours.....	3	7.17	42.3	3.40	2.8- 4.4	0.50	1.25-5.55
9 hours.....	3	7.33	43.3	4.53	3.8- 5.6	0.54	2.21-6.85
24 hours.....	3	6.73	35.3	2.87	2.6- 3.2	0.18	2.10-3.64
12° C.:							
0 hour.....	3	7.13	43.3	31.67	22.8-37.4	4.50	--
1 hour.....	3	7.30	40.7	6.80	6.0- 8.2	0.70	3.79-9.81
3 hours.....	3	6.67	30.7	1.00	0.8- 1.2	0.11	0.53-1.47
6 hours.....	3	7.73	51.7	0.93	0.8- 1.0	0.07	0.63-1.23
9 hours.....	3	7.18	40.3	1.40	1.0- 1.8	0.23	0.41-2.39
24 hours.....	3	6.93	45.0	0.87	0.8- 1.0	0.05	0.65-1.09
17° C.:							
0 hour.....	3	7.83	53.3	14.87	14.0-16.6	0.96	--
6 hours.....	3	7.57	48.7	2.27	2.0- 2.6	0.18	1.50-3.04
9 hours.....	3	7.40	41.7	1.80	1.6- 2.0	0.11	1.33-3.27
24 hours.....	3	7.46	43.7	1.40	1.2- 1.6	0.11	0.93-1.87

three temperatures were 0 to 4 p.p.m. above backgrounds, but the overlap of confidence intervals suggests that little, if any, residue remains beyond 9 hours (table 11). The residues dissipated more rapidly at 12°, and no significant amount persisted after 3 hours. The dissipation was intermediate and gradual at 17° and slowest at 7°.

DISCUSSION

Residues of MS-222 dissipated rapidly in rainbow trout, brown trout, brook trout, and lake trout within 1 to 6 hours after withdrawal from deep anesthesia (fig. 2). Regardless of species and temperature, there was very little difference in muscle between the residues of MS-222 and quantities of background amines at 9 to 24 hours. The higher concentrations of residues in all fish were measured at 0-hour withdrawal, and it was here that

differences were greater among individuals and species.

Rainbow trout and brown trout had higher initial residues than brook trout and lake trout despite the fact that the latter species were exposed to higher concentrations of MS-222. The chars, however, had the briefer exposures.

The chars may have oral and gill membranes which are less permeable than those of trouts to MS-222. The lake trout appear to be less able than brook trout to metabolize or detoxify the drug because they showed less tolerance and higher residues. Also, the anesthetic is more toxic to lake trout than brook trout (Marking, 1966).

The mineral content of water affected the mean time to anesthetize fish and the amount of residue in them. The 0-hour residues in

fish exposed in soft water (10 p.p.m. total hardness) were triple those in fish in hard water (180 p.p.m. total hardness). The fish in soft water, however, were exposed twice as long as in harder water. At the end of 24 hours, there were no significant differences in residues.

Acetylated residues in fish were not unexpected when one considers the similarity of MS-222 to *p*-aminobenzoic acid and the sulfonamides. These aromatic amines were probably acetylated in the liver or kidney and excreted (Hawk et al., 1954; Jessop, 1961). We do not know whether the conjugation of MS-222 with acetic acid in fish is a significant mechanism of detoxification or deactivation.

In general, the residues of MS-222 in fish at 7° and 12° were greater at 0-hour withdrawal than at 17° (figure 2). They persisted longer at 7°, but the concentrations of anesthetic solution and the durations of anesthesia also were greater at this temperature. The shorter exposures to lower concentrations of drug at 17° resulted in less but more consistent residues in all species at 0-hour withdrawal.

CONCLUSIONS

Residues of MS-222 occurred in the blood, muscle, liver, and kidney of deeply anesthetized rainbow trout. They dissipated rapidly within 1 to 6 hours, but those in liver and kidney were masked by background substances which interfered with the modified Bratton-Marshall colorimetric method.

The residues of MS-222 in muscle varied greatly at 0-hour withdrawal among anesthetized rainbow trout, brown trout, brook trout, and lake trout. They were higher in the trouts than in the chars. Regardless of species and temperature, there was very little difference between the residues of MS-222 and background amines in muscle at 9 to 24 hours after withdrawal. The residues of anesthetic in muscle of fish at 7° and 12° C. were greater

at 0-hour withdrawal than at 17°. Residues in muscle persisted longer at 7° than at warmer temperatures.

The 0-hour residues of drug in muscle of fish exposed in soft water were triple those of fish in hard water. At the end of 24 hours, however, there were no significant differences in residues.

SUMMARY

The residues of MS-222 in selected tissues of rainbow trout, brown trout, brook trout, and lake trout at 7°, 12°, and 17° C. and in waters of various hardnesses were measured by means of the modified Bratton-Marshall colorimetric method. The concentrations of drug in the blood, muscle, liver, and kidney of deeply anesthetized rainbow trout dissipated rapidly within 1 to 6 hours. Those in the liver and kidney were masked by background substances which interfered with the colorimetric method.

The amounts of MS-222 including background in muscle of the four species at 0-hour withdrawal were 6 to 72 p.p.m. The mean concentrations at 7°, 12°, and 17° C. were 18 to 42 p.p.m. in rainbow trout, 13 to 44 p.p.m. in brown trout, 15 to 28 p.p.m. in brook trout, and 15 to 32 p.p.m. in lake trout. At 24 hours after withdrawal from the anesthetic, the mean concentrations including background were 0.8 to 1.2 p.p.m. in rainbows, 0.8 to 1.4 in browns, 0.8 to 1.9 in brook trout, and 0.9 to 2.9 p.p.m. in lake trout. Actually, there was little difference between the residues of drug and background amines at 9 to 24 hours after withdrawal.

At 7° and 12° the concentrations of residue including background were generally above 20 p.p.m. at 0-hour withdrawal, whereas at 17° they were below 20 p.p.m. The residues persisted longer at 7° than at the warmer temperatures. At 9 to 24 hours the concentrations of drug at the three temperatures approached those of background amines in controls.

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1954. Practical physiological chemistry. 13th ed. McGraw-Hill Book Co., New York. 1439 p.
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1961. Fearon's introduction to biochemistry. 4th ed. Academic Press, New York. 473 p.
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1964. Investigations in Fish Control: 1. Laboratories and methods for screening fish-control chemicals. U.S. Bureau of Sport Fisheries and Wildlife, Circular 185. 15 p.
- Marking, Leif L.
1966. Investigations in Fish Control: 12. The toxicity of MS-222 to selected fishes: U.S. Bureau of Sport Fisheries and Wildlife, Resource Publication 18.
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1966. Investigations in Fish Control: 14. Method for determining MS-222 residues in fish. U.S. Bureau of Sport Fisheries and Wildlife, Resource Publication 20.

16. Annotated Bibliography on MS-222

By Richard A. Schoettger



United States Department of the Interior, Stewart L. Udall, *Secretary*
Stanley A. Cain, *Assistant Secretary for Fish and Wildlife and Parks*
Fish and Wildlife Service, Clarence F. Pautzke, *Commissioner*
Bureau of Sport Fisheries and Wildlife, John S. Gottschalk, *Director*
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ANNOTATED BIBLIOGRAPHY ON MS-222

By Richard A. Schoettger, Fishery Biologist
Bureau of Sport Fisheries and Wildlife
La Crosse, Wisconsin

Abstract.--This bibliography contains 86 selected references on uses of MS-222 on cold-blooded animals including fish and amphibians. Most of the references are annotated.

The Fish Control Laboratories at La Crosse, Wis., and Warm Springs, Ga., initiated studies in 1964 on the toxicity, efficacy, and residues of MS-222 in fish. The data were required by the U.S. Food and Drug Administration in order to clear the drug for continued use. In connection with the studies, a substantial bibliography on uses of MS-222 was compiled. A considerable number of the more useful references were annotated.

Some recognized references on MS-222 were not available conveniently for review and they have been included by title only.

BIBLIOGRAPHY

Allison, Leonard N.

1961. The effect of tricaine methanesulfonate (M.S. 222) on motility of brook trout sperm. *Progressive Fish-Culturist*, vol. 23, no. 1, p. 46-48.

The spermatozoa of brook trout remained motile less than 10 seconds in a concentration of 18.9 p.p.m. of MS-222. Allison recommended that the drug should not contact reproductive products during spawn-taking.

Bailey, Merryll M.

1965. Lake trout fin-clipping rates at two national fish hatcheries. *Progressive Fish-Culturist*, vol. 27, no. 3, p. 169-170.

MS-222 was used as an anesthetic during the fin-clipping of more than 3.5 million lake

trout. The fish ranged from 4.1 to 7.9 inches long and were young-of-the-year to 3-year-olds. One fin each was removed from 2 million fish, and 2 fins each were clipped on 1.5 million. Postmarking mortality usually was 0.1 to 0.2 percent; it increased with temperatures over 65° F. Cooling the solutions and reducing the concentration of MS-222 lowered mortality.

Ball, J. N., and P. N. Cowen.

1959. Urethane as a carcinogen and as an anaesthetic for fishes. *Nature*, vol. 184, p. 370.

Urethane is carcinogenic in the lung and other tissues of mice and rats. It does not produce tumors in rabbits, chickens, and guinea-pigs, but carcinogenicity may vary with species (and strain). It has a leucopenic effect on humans and can be absorbed from the skin.

MS-222 has not been reported to be carcinogenic. Its effectiveness on fish and amphibians varies with species, size of the animal, and temperature. Suitable concentrations are determined empirically for every species and situation.

Baudin, Louis.

- 1932a. Action de la tricaïne sur la consommation d'oxygène de Carassius auratus. *Comptes rendus des Séances de la Société de biologie*, vol. 109, p. 731-733.

A 1:1,000 solution of MS-222 anesthetized goldfish within 1 to 2 minutes. Observations

on the circulation of blood in the fins revealed a slower than normal rate with prolonged exposure to the anesthetic. A concentration of 1:20,000 produced a level of anesthesia in 30 to 60 minutes at 16.5° C. which could be maintained for 24 hours without apparent damage. The rate of oxygen consumption was depressed to 50 percent of normal after 1 hour and to 25 percent after 11 hours of exposure. The respiratory rate returned to normal within 2 hours after the fish were placed in fresh water. Above 16° C., narcosis was incomplete and the reduction of oxygen consumption was less. Below this temperature, a longer period was required for anesthesia and, for a fixed period, the rate of oxygen consumption was higher than at 16.5° C.

1932b. Action de la tricaïne sur le quotient respiratoire de Carassius auratus. Ibid., p. 1081-1083.

1932c. Perte de la sensibilité à la dépression chez les poissons anesthésiés à la tricaïne. Ibid., vol. 110, p. 151-153.

Oxygen consumption by goldfish was increased at lower atmospheric pressures. The rates for fish which were anesthetized with 1:20,000 of MS-222 were relatively low under conditions of both normal and reduced pressure. The sensitivity of the fish to reduced pressure was not completely suppressed by concentrations of 1:30,000 and 1:40,000.

1932d. Respiration du poisson (Carassius auratus) anesthésié à la tricaïne et soumes à une élévation brusque de température. Ibid., p. 235-237.

1934. Action de la tricaïne sur le sang des poissons. Ibid., vol. 115, p. 510-512.

A 1-percent solution of MS-222 anesthetized several species of fish, including goldfish and perch, within 1 minute. There were no effects on the erythrocytes, erythrocyte count, or oxygen capacity of the blood. The oxygen saturation of the blood was near zero, and the carbon dioxide level was slightly in excess. Lower concentrations of the drug and longer exposures induced exaggerated respiratory movement which increased the oxygen saturation and lowered

the carbon dioxide level. The author concluded that MS-222 may excite respiratory centers of the brain, or interfere with exchanges of gases, thereby causing anoxia and hyperventilation. With complete narcosis, the blood showed signs of asphyxia and there was a slowing of the circulation which contributed to reductions in erythrocyte numbers and oxygen capacity of the blood.

Bell, Gordon R.

1964. A guide to the properties, characteristics, and uses of some general anaesthetics for fish. Fisheries Research Board of Canada, Bulletin 148, 4 p.

Properties of eleven chemicals used as fish anesthetics are summarized. The chemicals are carbon dioxide, chloral hydrate, chloretone, ether, methyl pentynol, MS-222, phenoxyethanol, quinaldine, sodium amytal, tribromoethanol, and tertiary amyl alcohol. The common and chemical names of the chemicals, manufacturers, costs, solubilities, stabilities, hazards, toxicities, emergency treatments, approximate dosages, precautions, behavioral effects, uses, and modes of action are included. Concentrations of MS-222 from 1:9,000 to 1:4,500 were recommended for brief exposures. The generally useful range is between 1:25,000 to 1:12,500.

Black, Edgar C., and Anne R. Connor.

1964. Effects of MS 222 on glycogen and lactate levels in rainbow trout (Salmo gairdneri). Journal of the Fisheries Research Board of Canada, vol. 21, no. 6, p. 1539-1542.

A concentration of 0.5 g. of MS-222 per gal. anesthetized trout within 60 seconds. The fish showed signs of initial stimulation. The dosage might have been fatal with prolonged exposure. The hemoglobin, blood and muscle lactate, and muscle glycogen levels were similar in anesthetized and control fish.

Blahm, T. H.

1961. Effect of tricaine methanesulfonate on oxygen consumption of juvenile sockeye salmon. Transactions of the American Fisheries Society, vol. 90, no. 2, p. 226-227.

MS-222 uniformly reduced the oxygen consumption of both large (160-180 mm. fork

length) and small (60-80 mm. fork length) sockeye salmon. The fish were anesthetized by 1:20,000 in a constant flow respirometer.

Bové, Frank J.

1962. MS-222 Sandoz--the anaesthetic of choice for fish and other cold-blooded organisms. Sandoz News, no. 3, 12 p.

The author summarizes the history of MS-222 from its use as a local anesthetic in human medicine to its current status as a general anesthetic for fish, amphibians, and other cold-blooded forms. His personal communications with various investigators reveal that concentrations ranging from 1:3,785 to 1:17,500 have been used in measuring and weighing, marking, and spawning of various salmonids. A concentration of 1:12,000 seemed to be most popular. Solutions containing approximately 1:3,000 of MS-222 were effective on tropical fish, goldfish, bluegills, largemouth bass, and bullheads.

Effective dosages for amphibians ranged from 1:250 to 1:20,000, but 1:1,000 to 1:3,000 were preferred. Specimens with gills and those undergoing metamorphosis were more sensitive to the anesthetic.

Butler, Robert L.

1957. The development of a vinyl plastic subcutaneous tag for trout. California Fish and Game, vol. 43, no. 3, p. 201-212.

MS-222 at 0.5 g./gal. prepared fish for the operation within 30 seconds at 50° F. The fish were exposed no longer than 3 minutes. As temperature increases, it is necessary to reduce the exposure time or the concentration. The drug had no effect on feeding by the fish after they recovered from anesthetization.

Campbell, G. D., and D. H. Davies.

1963. Effect of ethyl m-aminobenzoate (MS-222) on the elasmobranch electrocardiograph. Nature, vol. 198, no. 4877, p. 302.

MS-222 caused coincidental decreases in the pulse and respiratory rates of stingrays indicating a neurological relationship between the cardiac and respiratory centers in the brain. The drug did not affect the ECG complex.

Christensen, K.

1931. Effect of castration on the secondary sex characters of males and females of Rana pipiens. Anatomical Record, vol. 48, p. 241.

Collins, James L., and Andrew H. Hulsey.

1963. Hauling mortality of threadfin shad reduced with M.S. 222 and salt. Progressive Fish-Culturist, vol. 25, no. 2, p. 105-106.

A combination of 0.5-percent salt and MS-222 equivalent to 1 g./12 gal. facilitated hauling of more than 200,000 fish with a survival of 95 percent.

Copenhaver, W. M.

1939. Initiation of beat and intrinsic contraction rates in the different parts of the Amblystoma heart. Experimental Zoology, vol. 80, p. 139.

Crawford, Bruce, and Andrew Hulsey.

1963. Effects of M.S. 222 on the spawning of channel catfish. Progressive Fish-Culturist, vol. 25, no. 4, p. 214.

The anesthetization of adult channel catfish with MS-222 at a rate of 4 g./8 gal. did not affect the success of spawning nor the viability of fry. The fish were narcotized for sexing and placement in spawning pens.

Dollar, Alexander M.

1963. Air transportation of living rainbow trout. Progressive Fish-Culturist, vol. 25, no. 3, p. 167-168.

The fish were placed in lake water containing MS-222 at a concentration of 1:100,000. The water was cooled gradually over a 3-hour period at 33° F. The fish and water were transferred to a plastic bag and packed in ice. Oxygen was added to the bags. The fish survived in the sealed containers for 24 hours.

Eisler, Ronald, and Tadeusz Backiel.

1960. Narcotization of chinook salmon fingerlings with tricaine methanesulfonate (M.S. 222). Transactions of the American Fisheries Society, vol. 89, no. 2, p. 164-167.

A concentration of 1:33,000 effectively anesthetized this species within 5 minutes.

The rate of narcotization was dependent on concentration. Recovery time increased with exposures up to an hour, but declined with longer exposure. The drug was equally active in fresh and salt water. The authors reviewed concentrations of MS-222 which have been used to anesthetize various species of fish.

Ellis, Robert J.

1964. The effect of confinement on blood lactate levels in chinook and coho salmon. Research Briefs, Fish Commission of Oregon, vol. 10, no. 1, p. 28-34

The lactic acid levels in the blood of troll-caught salmon declined more rapidly in those individuals which were held in solutions containing MS-222 at a concentration of 1:150,000.

Ewing, Ann.

1965. Current U.S. patents. A method for marking fish by which scales are transplanted painlessly from one part of the body to another has been patented. Science News Letter, vol. 87, no. 15, p. 228.

Dr. Louis Levy and Miss Carol A. De Fusco (1965) have patented a method of marking fish by replacing scales of one color from one part of the body with those of contrasting color from a different area. The fish are anesthetized during the operation with 50 to 100 p.p.m. of MS-222.

The technique was successful on goldfish, carp, blue acara, blackspot barb, and guppies, and has been used to measure the effects of drugs on the rejection time of scales from different fish.

Friddle, S. B., and S. F. Snieszko.

1950. Effect of tricaine methanesulfonate on the determination of sulfonamides. Science, vol. 112, no. 2902, p. 181-182.

Concentrations of 2 to 4 mg.%, as sulfamerazine, were detected in the tissues of trout which should not have contained the sulfa drug. The fish had been anesthetized for 1 minute in a 1:5,000 solution of MS-222. This anesthetic, or others with similar molecular structures, should not be used whenever they may interfere with the colorimetric test for sulfonamides.

Fromm, Paul O.

1958. A method for measuring the oxygen consumption of fish. Progressive Fish-Culturist, vol. 20, no. 3, p. 137-139.

MS-222 is used to immobilize fish for length and weight measurements before they are placed in a respirometer. A concentration of 0.03 percent produces anesthesia within 30 to 45 seconds. The drug apparently has no lasting effect on the general metabolic rate.

Gebhards, Stacy V.

1965. Transport of juvenile trout in sealed containers. Progressive Fish-Culturist, vol. 27, no. 1, p. 31-36.

The use of sedating levels of MS-222 did not increase the loading density or survival of rainbow trout in sealed containers. Greater survival was associated with the starvation period prior to loading.

Gilbert, P. W., and F. G. Wood.

1957. Methods of anaesthetizing large sharks and rays safely and rapidly. Science, vol. 126, p. 212.

A 1:1,000 solution of MS-222 is sprayed into mouth, spiracles, or gill exits by means of a hand sprayer, such as a water pistol. Sharks as large as 400 pounds are anesthetized in a minute or less and may be handled, out of water, for 5 to 30 minutes. The volume of anesthetic solution required to anesthetize fish varies with the size of the individuals.

Glücksohn, Salome.

1932. Äussere Entwicklung der Extremitäten und Stadieneinteilung der Larvenperiode von Triton taeniatus Leyd. und Triton cristatus Laur. W. Roux' Archiv für Entwicklungsmechanik der Organismen, vol. 125, p. 341-405.

Metamorphosing salamanders were anesthetized with 1:3,000 of MS-222 for 30 minutes a day. The growth of treated specimens was somewhat less than controls, but the former were proportioned normally.

Goodrich, H. B., and R. Nichols.

1931. The development and regeneration of color pattern in Brachydanio rerio. Anatomical Record, vol. 51, p. 513.

Gossington, Robert.

1957. An aid to fish handling--tricaine. Aquarium Journal, vol. 28, no. 9, p. 318-321.

Fish could be anesthetized safely with MS-222 in concentrations of 0.24 to 0.32 g./gal. Larger specimens required somewhat higher concentrations. Live-bearers were generally more resistant than egg-layers. The drug reduced the chances of injury to fish from thrashing during shipment. Under anesthesia, Siamese fighting fish could be shipped together in a single container.

Hadian, Z., and M. S. Dunn.

1938. Localisation in the oculomotor nuclei of the goldfish. Journal of Comparative Neurology, vol. 68, p. 191.

Hublou, Wallace F.

1957. A method of using an anesthetic in marking fins. Progressive Fish-Culturist, vol. 19, no. 1, p. 40-43.

MS-222 was used in a recirculating system to fin-clip fingerling salmon and steelhead trout. The marking rate was improved by 49.5 percent with the use of an anesthetic. Advantages of the system included better marks, lower rate of injury, reduction of worker fatigue, and saving in time and money.

Johnson, Harlan E., and J. M. Shelton.

1958. Marking chinook salmon fry. Progressive Fish-Culturist, vol. 20, no. 4, p. 183-185.

Groups of 10 to 20 individuals were caught in a small net and placed in a 1:7,500 solution of MS-222. Fin-clipping began when all the fish were on their sides and after the net had been placed in fresh water. The last fish was marked just before it recovered. Approximately 487,000 fry were marked at a cost of \$50.

Karczmar, Alexander G., and Theodore Koppanyi.

1948. Action of central nervous system depressants at different growth periods of salamander (*Amblystoma punctatum*) larvae. Federation Proceedings, vol. 7, p. 231-232.

Larvae of different ages were immersed in a 1:7,500 solution of MS-222, and the times for anesthesia were recorded. Paraldehyde, ethyl alcohol, sodium barbital, nembutal, chloral hydrate, and chloretone were also tested. Anesthesia with MS-222 was more rapid in older and larger individuals.

Klontz, George W.

1964. Anesthesia of fishes. From: Proceedings of the Symposium on Experimental Animal Anesthesiology, Brooks Air Force Base, December 14-16, 13 p.

The efficacy and characteristics of 14 methods which are used to anesthetize fish were discussed.

Concentrations of 25 to 35 p.p.m. of MS-222 are recommended for transporting fish; 50 to 100 p.p.m. are used to induce deep anesthesia. In general, induction time requires 1 to 3 minutes of exposure and the fish recover, in fresh water, within 3 to 15 minutes. Fish which are repeatedly exposed to MS-222 showed a slight increase in tolerance which is corrected by raising the concentration slightly. The drug appears to be toxic to those fish which are treated in salt water and in direct sunlight.

Knight, Alexis E.

1964. Intracellular hemoglobin crystallization in two centrarchids, the largemouth bass and the bluegill. Progressive Fish-Culturist, vol. 26, no. 3, p. 115-117.

A 1:5,000 solution of MS-222 was used to narcotize fish during the collection of blood samples.

Koppanyi, Theodore, and Alexander G. Karczmar.

1948. Comparison of anesthetic action of acetanilid, tricaine (MS-222) and aliphatic depressants. Federation Proceedings, vol. 17, p. 234.

The action of acetanilid on salamander larvae was independent of larval stage. Subanesthetic dosages of the drug acted additively with subanesthetic levels of MS-222, nembutal, and chloretone. The anesthetic effects of chloretone, MS-222, alcohol, paraldehyde, and acetanilid were reversed rapidly.

Lemarque, Pierre.

1964. Anesthésie et transport. Bull. Inf. Cons. Sup. Pêche, vol. 55, p. 5-9.

MS-222 may be used in concentrations of 1:10,000 to 1:50,000 to anesthetize fish before they are placed in plastic bags at a loading level of 1 kilogram of fish per 1 to 2 liters of water. The bags were filled with oxygen. Dosages of 1:100,000 were recommended for the tranquilization of fish in transportation tanks.

Larsen, Howard N.

1964. Comparison of various methods of hemoglobin determination of catfish blood. Progressive Fish-Culturist, vol. 26, no. 1, p. 11-15.

The fish were anesthetized in a 1:5,000 solution of MS-222 to facilitate the collection of blood samples.

Levy, Louis Encino, and Carol A. DeFusco.

1965. Identification of scaly teleosts. U.S. Patent Office, Patent No. 3,174,458. 3 p.

Lumb, William V.

1963. Small animal anesthesia. Chapter: Anesthesia of laboratory and zoo animals, p. 269-310. Lea and Febiger, Philadelphia. 420 p.

MS-222 is used to immobilize fish and other cold-blooded animals by completely bathing small subjects, by gill spraying in large fish, or by injection in large animals. Concentrations of 0.5 to 1.0 grams of drug per gallon are used for most teleosts and the temperature is maintained at 40° to 60° F. Repeated use of anesthetic solutions reduces their efficacy. Longer anesthesia or sedation can be maintained with lower concentrations. The drug can also be used in treatment of fungus infections and other localized diseases on pet or ornamental fish.

The uses of ether, sodium amytal, carbon dioxide, urethane, and cresol in anesthetizing fish are discussed.

Marking, Leif L.

1966. Investigations in Fish Control. 12. Toxicity of MS-222 to selected fishes. U.S. Bureau of Sport Fisheries and Wildlife, Resource Publication 18.

The 24-hour LC₅₀ concentrations of MS-222 for various species of fish, at 12° C., were found to be: rainbow trout, 39.0 to 52.0 p.p.m.; brown trout, 38.5 to 45.6 p.p.m.; brook trout, 50.7 to 52.2 p.p.m.; lake trout, 33.8 to 39.8 p.p.m.; northern pike, 56.0 p.p.m.; bluegill, 45.7 to 46.9 p.p.m.; largemouth bass, 42.0 to 61.5 p.p.m.; and walleye, 49.0 p.p.m. The results indicated that exposures of MS-222 for 24 to 96 hours had no significant effect on the toxicity of the chemical.

In general, MS-222 was more toxic to smaller individuals, and at higher temperatures. Water hardness had little effect on toxicity.

The safety index of MS-222 for rainbow trout was determined by comparisons of the LC₅₀ and EC₅₀ concentrations after 15, 30, and 60 minutes of exposure in relatively soft and hard water. The indexes ranged from 1.7 to 2.0 and decreased slightly with exposure time. A comparison of the LC₁ and EC₉₉ for hard water gave an index of about 1.3 and 1.0 to 1.1 in soft water.

Maintenance of anesthesia in fish for 96 hours was not harmful. After the fish had been placed in fresh water and recovered, they fed as well as controls.

Martin, N. V., and D. C. Scott.

1959. Use of tricaine methanesulfonate (M.S. 222) in the transport of live fish without water. Progressive Fish-Culturist, vol. 21, no. 4, p. 183-184.

Hybrid trout were anesthetized in 60 p.p.m. of MS-222 and packed in layers of chipped ice and sphagnum moss. The fish were maintained under these conditions for 4 to 4.5 hours with little mortality.

McFarland, William N.

1959. A study of the effects of anesthetics on the behavior and physiology of fishes. Publications of the Institute of Marine Science, University of Texas, vol. 6, p. 23-55.

The anesthetic effects of 21 chemicals, including MS-222, were tested against Fundulus parvipinnis, Gambusia affinis, Paralabrax clathratus, and Girella nigricans. The behavioral changes induced in fish by anesthetics were classified into the following levels of anesthesia: sedation, loss of

equilibrium, loss of reflex reactivity and medullary collapse. Anesthesia in fish was compared to that in humans and was found to be a similar process involving sequential suppression of higher to lower central nervous centers.

The narcotic potencies of the various compounds increased with their molecular weights. MS-222 was rated as highly potent. The ratio of the dosages necessary to induce sedation and medullary collapse during a 12-hour period was 7.1.

Anesthesia with MS-222 was more rapid at 27° than at 12° C., but anesthesia did not progress as deeply at the lower temperature. Metabolic studies indicated that MS-222 was depleted, with time, at a greater rate than other anesthetics.

McFarland, William N.

1960. The use of anesthetics for the handling and the transport of fishes. California Fish and Game, vol. 46, no. 4, p. 407-431.

MS-222, tertiary amyl alcohol, and methylparafynol were suggested as beneficial for the induction of deep anesthesia because they act quickly and recovery is rapid. Recovery from anesthesia was complete provided respiratory movements had not ceased for more than a few minutes. MS-222 at 0.03 g./gal. induced loss of reflex in Fundulus parvipinnis within 1 hour. Higher concentrations were recommended for more rapid anesthesia; however, fish must be removed from the anesthetic after the desired stage of anesthesia has been induced.

MS-222 was not recommended for transporting fish. The drug failed to maintain a lowered rate of metabolism at higher temperatures.

It is advisable to pretreat fish in an anesthetic before transporting to reduce metabolic rates which may be stimulated due to handling.

McGovern, Beulah H., and Roberts Rugh.

1944. Efficacy of m-amino ethyl benzoate as an anesthetic for amphibian embryos. Proceedings of the Society for Experimental Biology and Medicine, vol. 57, p. 127-130.

Dosages of 1:3,000 did not affect the motility or fertility of frog spermatozoa. The eggs which were fertilized in this solution developed normally when the exposure did not exceed 1 hour. Longer exposures produced decreasing numbers of abnormal embryos as development progressed through gastrulation and neurulation. The older embryos withstood anesthesia for 24 hours; however, mortality of the embryos undergoing transition from external to internal gill respiration increased with immersions in MS-222 longer than 2 hours. The drug inhibited muscular action, but not ciliary activity. MS-222 was considered to be non-toxic to frog embryos within the exposure times adequate for surgical operations.

Meehan, William R., and L. Revet.

1962. The effect of tricaine methanesulfonate (M.S. 222) and/or chilled water on oxygen consumption of sockeye salmon fry. Progressive Fish-Culturist, vol. 24, no. 4, p. 185-187.

The most favorable conditions for survival of fish during transportation appeared to be uncrowded numbers of fish in water colder than that from which they were removed. The fish also survived well when uncrowded in their normal environmental water to which was added 0.1 g. of MS-222 per 4,000 ml. Unsatisfactory results were obtained with crowding, or when the fish were placed in solutions of MS-222 which were colder than their environmental water.

Meister, Alfred L., and Charles F. Ritzi.

1958. Effect of chloretone and MS-222 on eastern brook trout. Progressive Fish-Culturist, vol. 20, no. 3, p. 104-110.

MS-222 was considered superior to chloretone as an anesthetic for fishery use. The former had a wider range of practical field concentrations, lesser inhibitory effect on respiration, and was easier and more predictable for use in the field. Both drugs produced more rapid anesthetization when temperatures were increased. Anesthesia with MS-222 was induced within approximately 10 minutes by concentrations ranging from 1:5,000 to 1:15,000 at 37° to 39° F., and 1:5,000 to 1:25,000 at 48° to 51° F. A concentration of 1:1,000 produced respiratory

arrest in brook trout after 5 minutes, but continued exposure for 6 minutes was not fatal.

They observed that 22 to 35 pounds of brook trout, 36 to 86 pounds of salmon or 93 pounds of lake trout could be anesthetized per gram of MS-222.

Moss, D. D., and D. C. Scott.

1964. Respiratory metabolism of fat and lean channel catfish. *Progressive Fish-Culturist*, vol. 26, no. 1, p. 16-20.

MS-222 was used at the rate of 1 g./3.8 l. to anesthetize channel catfish for measurements of lengths and weights. The fish were placed in a respirometer and recovered from the anesthetic within 1 to 2 minutes. The oxygen consumption of fat fish was greater than that of thin fish at 25° C. At 30° C. the respiratory rates for both groups were similar.

Nelson, P. R.

1953. Use of three anesthetics on juvenile salmon and trout. *Progressive Fish-Culturist*, vol. 15, no. 2, p. 74.

MS-222, chlorobutanol, and urethane were used as aids in weighing and measuring coho salmon, red salmon, and Dolly Varden trout. MS-222 was used at the rate of 1:12,500 at 12° to 17° C. and effectively anesthetized the fish within several minutes. The concentration was increased slightly for fingerlings. A dosage of 1:10,000 caused 100 percent mortality.

Normandeau, Donald A.

1962. Microhematocrit values for some salmonids reared in New Hampshire. *Progressive Fish-Culturist*, vol. 24, no. 4, p. 172-176.

A solution containing 1:10,000 of MS-222 was used to anesthetize landlocked salmon, rainbow, brook, lake, and splake trout before collection of microhematocrits. The fish were anesthetized sufficiently within 1 minute. There was a significant relation of mean hematocrits to sampling date, but not to water temperature.

Parkhurst, Z. E., and M. A. Smith.

1957. Various drugs as aids in spawning rainbow trout. *Progressive Fish-Culturist*, vol. 19, no. 1, p. 39.

MS-222, sodium amytal, methyl pentynol, urethane, and chloretone were used to anesthetize rainbow trout.

A concentration of 264 p.p.m. of MS-222 induced complete anesthesia within 30 to 45 seconds. Longer exposures resulted in some mortality. The fish were in good condition 75 days after treatment. The hatching success of eggs from anesthetized and control fish was similar.

Methyl pentynol at 2,400 p.p.m. was effective within 3.5 minutes, a 0.5-percent solution of urethane in 2 minutes, and 400 p.p.m. of chloretone in 1.0 to 1.5 minutes. Sodium amytal, because of its slow action, had no practical value.

The experiments were conducted at a temperature of 43° F.

Phillips, Arthur M., Jr., Henry A. Podoliak, Donald R. Brockway, and Ray R. Vaughn.

1957. The nutrition of trout. Cortland Hatchery Report 26, New York Conservation Department, Fisheries Research Bulletin 21. 93 p.

The absorption of radioactive cobalt was elevated in brook trout which were narcotized with MS-222. It was suggested that there may be an adjustment of the osmotic processes of narcotized fish.

Pickford, Grace E.

1953. A study of the hypophysectomized male killifish, *Fundulus heteroclitus* (Linn.) Bulletin of the Bingham Oceanographic College, vol. 14, no. 2, p. 5-41.

1957. Methods of hypophysectomy in fishes. Appendix to: The physiology of the pituitary gland of fishes, by Grace E. Pickford and James W. Atz, p. 485-487. New York Zoological Society, New York. MS-222 is recommended over several other techniques and agents for anesthetizing fish during hypophysectomy. The drug gave excellent results with *Fundulus heteroclitus*, but suitable strengths must be determined for each species.

Piper, Robert G., and Robert F. Stephens.

1962. A comparative study of the blood of wild and hatchery reared lake trout.

Progressive Fish-Culturist, vol. 24, no. 2, p. 81-84.

Blood samples were collected by heart puncture after anesthetizing the trout in a 1:1,000 solution of MS-222. The hemoglobin levels and erythrocyte counts of the hatchery-reared and wild lake trout were similar.

Pulford, Earl F., and L. M. Woodall.

1963. An operculum marking experiment on juvenile chinook salmon. Research Briefs, Fish Commission of Oregon, vol. 9, no. 1, p. 30-36.

MS-222 was used as an anesthetic during marking of 1.5-inch salmon. There was little mortality of the fish which were maintained under observation for 112 days.

Randall, D. J.

1962. Effect of an anaesthetic on the heart and respiration of telost fish. Nature, vol. 195, no. 4840, p. 506.

Heart and respiratory rates were measured in tench exposed to 25 to 200 mg./l. of MS-222 at 17° C. The heart rate in undisturbed, control fish was 15 to 30 beats per minute. At a concentration of 33 p.p.m. of MS-222, the rate exceeded 50 per minute, and increased at higher dosages. The respiratory rate and amplitude also increased, but in a variable manner.

MS-222 probably acts on the heart via the parasympathetic nervous system since the direct effect of the drug on isolated and perfused hearts of tench, trout and roach decreased the beat frequency. Bilateral sectioning of the vagi of the fish, which were exposed to MS-222, resulted in a reduced heart rate.

Respiratory collapse occurred at 100 to 200 p.p.m. of MS-222.

Robinson, Clay.

1965. Those chasing-rainbows. U.S. Trout News, January-February, p. 5.

The anesthetic action of MS-222 varies according to water temperature and hardness.

Robertson, O. H.

1958. Accelerated development of testis after unilateral gonadectomy, with observations on normal testis of rainbow trout. U.S. Fish and Wildlife Service,

Fishery Bulletin, No. 127, vol. 158, p. 9-30.

MS-222 was used to anesthetize fish for gonadectomy and for length and weight measurements. A concentration of 1:20,000 induced adequate anesthesia in 2 to 3 minutes. The fish recovered rapidly, and no harmful effects were observed even after daily use for a number of weeks.

The operational technique included starvation of the fish for 48 hours and initial anesthesia in 1:20,000 of MS-222. The fish were placed on an operating board and then dipped into a 1:25,000 solution of the anesthetic.

The sequence of histological changes in gonadectomized fish and those in which laparotomy only was performed were the same as in normally maturing gonads.

Rodman, Duane T.

1963. Anesthetizing and air-transporting young white sturgeons. Progressive Fish-Culturist, vol. 25, no. 2, p. 71-78.

MS-222, tertiary amyl alcohol, and reduced temperature were employed in air-transporting young sturgeon. Shipments following use of two drugs were not successful. Fish exposed to a 1:40,000 solution of MS-222 survived for 30 to 48 hours but later died. A temperature of 40° F. provided adequate cold sedation which could be maintained during transport for approximately 30 hours with dry ice. The fish were shipped successfully by this method.

Rothlin, E.

1932. M.S. 222 (lösliches Anaesthesin), ein Narkotikum für Kaltblüter. Schweizerische Medizinische Wochenschrift, vol. 62, no. 45, p. 1042-1043.

MS-222 is a third as toxic to cold-blooded animals as novocaine and a tenth as toxic as cocaine. MS-222 was more efficacious in comparison with novocaine, strovain, alypin, tutokain, panthesin, kokaine, barokain, and eukain. A frog was completely anesthetized within 5 to 7 minutes in solutions of 1:1,000 to 1:2,000 of MS-222. Narcosis lasted several hours, and when the animal was placed in fresh water it recovered in 30 to 60 minutes. Studies with homologs of MS-222--allyl, isopropyl, n-butyl ester--gave no better results than the ethyl ester.

Rotmann, Eckhard.

1931. Die Rolle des Ektoderms und Mesoderms bei der Formbildung der Kiemen und Extremitäten von Triton. 1. Operation in Gastrulastadium. W. Roux' Archiv für Entwicklungsmechanik, vol. 124, p. 747-794.

There were no side or after effects of MS-222 on salamanders which were anesthetized by concentrations of 1:3,000 for 1 hour. Repeated anesthetization was not harmful.

Ryder, R. A.

1960. Comparative tagging returns employing three different anesthetics. Canadian Fish Culturist, no. 26, p. 23-25.

Ether, urethane, and MS-222 were used to anesthetize Stizostedion v. vitreum for tagging. After 2 years, approximately twice as many fish tagged with the help of MS-222 had been recovered as those tagged with the help of either of the other anesthetics.

Sakano, Ei-ichi.

1961. Anaesthetizing experiments of chum salmon fry with tricaine methanesulfonate (M.S. 222). Scientific Reports of the Hokkaido Salmon Hatchery, No. 16, p. 103-106.

Sandoz, M.

1920. Recherches expérimentales sur les anesthésiques locaux. 1. Préparations et propriétés physiologiques de la tricaine et de quelques-uns de ses dérivés, Bull. Soc. Vaud. Sc. Nat., vol. 53, p. 263-302.

Sandoz Pharmaceuticals

- (No date) The toxicity of MS-222 to fish and frogs. Sandoz Pharmaceuticals, Hanover, N.J. (Mimeo) 2 p.

The 30-minute LC₅₀ of MS-222 for frogs was 1:160.

A 1:12,200 concentration produced 50-percent mortality of young trout in 15 minutes. The maximal tolerated concentration (LC₁) for trout was 1:15,900 and a 1:25,000 solution induced anesthesia in 99 percent of the individuals (EC₉₉) within 3 to 4 minutes. These data gave a therapeutic index for MS-222 of 1.57.

A 10-percent solution of MS-222 which was stored at room temperature showed no loss in activity after 3 days. After 10 days there was a reduction in activity of about 5 percent. No difference was noted between solutions protected or not protected against light.

Sandoz Pharmaceuticals

- (No date) M.S. 222-Sandoz, the anesthetic of choice in work with cold-blooded animals. Sandoz Pharmaceuticals, Hanover, N.J., Technical Bulletin. 10 p.

Concentrations of 0.5 to 1.0 g./gal. anesthetic silver salmon, sockeye salmon, lake trout, brown trout, and largemouth and smallmouth bass within 2 to 4 minutes at 40° to 60° F. A dosage of 0.25 to 1.0 g./gal. is recommended for rainbow trout.

A concentration of 0.14 g./gal. is recommended for tranquilizing bait fish during transport. Levels up to 0.32 g./gal. are effective for various tropical species.

Sato, T.

1930. Beiträge zur Analyse de Wolff'schen Linsen regeneration. Wilh. Roux' Archiv für Entwicklungs mechanik d. Organismen, vol. 122, p. 451.

Schiffman, R. H., and P. O. Fromm.

1959. Measurement of some physiological parameters in rainbow trout (Salmo gairdnerii). Canadian Journal of Zoology, vol. 37, p. 25-32.

Rainbow trout were anesthetized in 300 p.p.m. of MS-222, placed on their backs and their hearts exposed. Blood samples were collected by cardiac puncture. The samples were divided for measurements of hematocrit, hemoglobin, and erythrocyte count and size. In addition, weights of organs and body water and volumes of blood and plasma were determined.

Schoettger, Richard A., and Arnold M. Julin.

1966. Investigations in Fish Control: 13. Efficacy of MS-222 as an anesthetic on four salmonids. U.S. Bureau of Sport Fisheries and Wildlife, Resource Publication 19.

Concentrations of MS-222 ranging from 80 to 135 p.p.m. effectively anesthetized rainbow,

brown, brook and lake trout within 3 minutes at 7° to 17° C. The fish were exposed safely to these concentrations for 4 to 12 minutes. Dosages of 50 to 60 p.p.m. induced anesthesia within 15 minutes, and 15 to 30 p.p.m. were effective for sedation. The effective concentrations and exposure times were inversely related to temperature; however, the efficacy of sedating concentrations declined with time at 17° C. The narcotic action of the drug was reversible, provided the fish were removed from the anesthetic prior to the cessation of respiratory activity.

The efficacy of MS-222 was not affected significantly by size of fish or pH of the solution. Anesthetic solutions with hardnesses of 10 p.p.m. were less effective than those containing 35 to 180 p.p.m. total hardness, but the fish treated in soft water recovered sooner.

The exposure tolerances of fish which were repeatedly anesthetized with MS-222 were slightly greater than those which were unexposed previously.

Trout sedated in closed systems at 12° C. reduced their rate of oxygen consumption by 30 percent; the rate was not depressed significantly in open systems.

Serfaty, A., R. Labat, and R. Quillier.

1959. Les réactions cardiaques chez la carpe (*Cyprinus carpio*) au cours d'une anesthésie prolongée. *Hydrobiologia*, vol. 13, p. 144-151.

Anesthetizing carp with 100 p.p.m. of MS-222 caused a primary and secondary tachycardia. The first was believed to be correlated with encephalic penetration of the drug, and the second with a reduction of vagal tonus and lack of oxygen. Eventually, auriculoventricular dissociation occurred which was attributed to damage of the intracardiac system.

Shelton, G., and D. J. Randall.

1962. The relationship between heart beat and respiration in teleost fish. *Comparative Biochemistry and Physiology*, vol. 7, p. 237-250.

The heart and respiratory rates of tench were increased by anesthetizing them in 80 to 200 p.p.m. of MS-222. After 12 minutes in a 200 p.p.m. solution, the fish stopped breathing and the heart rate fell to a level

similar to that of unanesthetized individuals. The heart beat and breathing became absolutely synchronized in animals lightly anesthetized in MS-222. The direct effect of MS-222 on isolated hearts was to decrease the beat rate at 15 p.p.m. and decrease beat amplitude above 30 to 60 p.p.m. Since fish have no sympathetic innervation of the heart, the authors suggested the presence of some cardioaccelerator fibers in the vagus nerve.

Smith, Lloyd L., Jr., Robert H. Kramer, and J. Cameron MacLeod.

1965. Effects of pulpwood fibers on fathead minnows and walleye fingerlings. *Journal, Water Pollution Control Federation*, vol. 37, no. 1, p. 130-140.

A concentration of 1,000 p.p.m. of MS-222 rapidly anesthetized fathead minnows for the measurement of hematocrits. The hematocrits of treated and control fish were not significantly different. The quantity of blood obtained from anesthetized individuals was 17 percent less than controls and probably indicates a reduced rate of circulation.

Smith, Lynwood S., and Gordon R. Bell.

1964. A technique for prolonged blood sampling in free-swimming salmon. *Journal of the Fisheries Research Board of Canada*, vol. 21, no. 4, p. 711-717.

The dorsal aortas of pink and sockeye salmon were cannulated to permit the sampling of blood over extended periods. The fish were anesthetized during the operation by irrigating the gills in a 1:15,000 solution of MS-222 using a pump recycling system. The cannula was attached to a length of polyethylene tubing which extended dorsal from the roof of the mouth to above the snout.

Snieszko, S. F.

1960. Microhematocrit as a tool in fishery research and management. U.S. Fish and Wildlife Service, Special Scientific Report--Fisheries No. 341. 15 p.

A technique for measuring the hematocrit of fish was described. The fish were anesthetized for approximately 1 minute in a 1:2,000 solution of MS-222.

Steinbrecht, Karl.

1957. Narkose von Fischen. Die Aquarien- und Terrarien-Zeitschrift, vol. 10, no. 11, p. 305-306.

There were no abnormalities of offspring from guppies which were frequently anesthetized in a 1:2,000 solution of MS-222 following fertilization.

Steucke, Erwin, W., Jr., and Charles R. Atherton.

1965. Use of microhematocrit values to sex largemouth bass. Progressive Fish-Culturist, vol. 27, no. 2, p. 87-90.

MS-222 at 1:3,000 was used to anesthetize largemouth bass. They were narcotized in about 2 minutes.

Thompson, R. B.

1959. Tricaine methanesulfonate (M.S. 222) in transport of cutthroat trout. Progressive Fish-Culturist, vol. 21, no. 2, p. 96.

Young cutthroat trout were transported successfully in plastic bags which contained oxygen and a 1:40,000 solution of MS-222. The temperature was reduced by packing the bags in ice.

One-quart, plastic food boxes were tested in place of plastic bags. Approximately 500 individuals were added to each box which was half filled with the 1:40,000 solution of anesthetic. Oxygen was not used in these tests, but the containers were packed in ice. The fish were released after 3 hours, and only 19 out of 2,000 failed to recover from anesthesia.

Villwock, W.

1958. Narkose bei Fischen. Aquarien-Terrarien-Zeitschrift, vol. 11, p. 28.

Walker, Charles R., and Richard A. Schoettger.

1966. Investigations in Fish Control: 15. Residues of MS-222 in four salmonids following anesthesia. U.S. Bureau of Sport Fisheries and Wildlife, Resource Publication 21.

Residues of MS-222 were measured in rainbow, brown, brook, and lake trout. The fish were anesthetized to medullary collapse in concentrations of 80 to 135 p.p.m., depend-

ing on species and temperature. Residues ranged from 6 to 72 p.p.m. in the muscle tissues of fish at the time of medullary collapse. The levels in fish which had recovered in freshwater at 12° C. declined rapidly within 3 hours and approached background values after 6 to 9 hours. A slower dissipation of residues occurred in tests at 7° and 17° C. The total residue including background did not exceed 5 p.p.m. at the end of 9 hours, or 3 p.p.m. after 24 hours for all species at all temperatures.

Residues of MS-222 in the blood, liver, and kidney of rainbow trout declined in a pattern similar to that for muscle.

1966. Investigations in Fish Control: 14.

Method for determining MS-222 residues in fish. U.S. Bureau of Sport Fisheries and Wildlife, Resource Publication 20.

The Bratton-Marshall method is sensitive for primary aromatic amines such as sulfa drugs. It was modified to detect MS-222.

Selected tissues from anesthetized trout were processed to extract the drug. The recovery of known amounts in muscle, blood, kidney, and liver ranged from 90 to 112 percent. The concentrations of interfering amines were evaluated.

The acute oral LD₅₀ of MS-222 to laboratory rats is between 5 and 10 g./k. This indicates a relatively low toxicity of MS-222 to mammals.

Watson, John E.

1961. Tricaine methanesulfonate as an anesthetic for herring. Progressive Fish-Culturist, vol. 23, no. 4, p. 174.

MS-222 was tested in sea water as an anesthetic for herring. The fish were anesthetized within 8 minutes by a solution of 1:20,000 at 8° C. They recovered in approximately 8 minutes after removal to fresh seawater. Their prolonged exposure to MS-222 for 3 to 4 minutes after complete anesthesia was usually lethal.

Webb, Robert T.

1954. Tricaine methanesulfonate (M.S. 222) as an anesthetic for some common pond fishes. Unpublished thesis. Alabama Polytechnic Institute, Auburn.

Webb, Robert T.

1958. Distribution of bluegill treated with tricaine methanesulfonate (M.S. 222). *Progressive Fish-Culturist*, vol. 20, no. 2, p. 69-72.

Tests were conducted to determine whether the application of MS-222 would increase the number or pounds of bluegills which could be carried in a distribution truck. A concentration of 0.1 g./gal. appeared to be the most promising. The results of the tests were inconclusive; successful tests could not be duplicated; and at times the control fish hauled as well or better than the drugged ones.

Witschi, E.

1927. Testis grafting in tadpoles of *Rana temporaria* L. and its bearing on the hor-

mone theory of sex determination. *Journal of Experimental Zoology*, vol. 47, p. 269.

Wood, E. M.

1956. Urethane as a carcinogen. *Progressive Fish-Culturist*, vol. 18, no. 3, p. 135-136.

Urethane induces lung tumors in mice which develop whether the drug is administered by injection, in drinking water, by nasal instillation, or from painting the skin.

An editorial comment accompanying this report indicated that MS-222 might be a suitable substitute for urethane.

17. MS-222 as an Anesthetic for Channel Catfish: Its Toxicity, Efficacy, and Muscle Residues

By Richard A. Schoettger, Charles R. Walker,
Leif L. Marking, and Arnold M. Julin



United States Department of the Interior, Stewart L. Udall, *Secretary*
Stanley A. Cain, *Assistant Secretary for Fish and Wildlife and Parks*
Fish and Wildlife Service, Clarence F. Pautzke, *Commissioner*
Bureau of Sport Fisheries and Wildlife, John S. Gottschalk, *Director*
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MS-222 AS AN ANESTHETIC FOR CHANNEL CATFISH: ITS TOXICITY, EFFICACY, AND MUSCLE RESIDUES

By Richard A. Schoettger, Fishery Biologist
Charles R. Walker and Leif L. Marking, Chemists
and Arnold M. Julin, Fishery Biologist
Bureau of Sport Fisheries and Wildlife
La Crosse, Wisconsin

Abstract.--MS-222 was tested as an anesthetic on channel catfish. Its acute toxicity is approximately 65 to 50 p.p.m. over periods of 24 to 96 hours. Anesthesia is induced within 2 minutes by concentrations above 100 to 140 p.p.m., and within 15 minutes by 70 p.p.m. Concentrations of 20 to 40 p.p.m. maintain sedation for 6 hours. Residues of MS-222 occur in muscles of anesthetized catfish, but decrease about 90 to 95 percent at 1 hour of withdrawal from the drug. Nine to 24 hours after withdrawal the residues decline to within the statistical variations of the background aromatic amines. The influences of duration of exposure, size of fish, temperature, and water quality on toxicity, efficacy, and residues are discussed.

MS-222 (tricaine methanesulfonate) is commonly employed to anesthetize fish during marking, spawning, and transporting. The drug has been used less commonly on channel catfish than on salmonids or centrarchids. In recent years the channel catfish, Ictalurus punctatus, has become highly regarded in many areas of the United States as a commercial and sport fish; it is being reared on rice-fish farms, in commercial hatcheries, and in farm ponds. We anticipate that a greater use of MS-222 will accompany increased effort to advance the culture and management of this species.

The U.S. Food and Drug Administration requires that certain disinfectants, antimicrobials, and anesthetics be cleared for their continued use on fish which may be consumed by people.

The information necessary for clearance of MS-222 as an anesthetic for channel catfish

includes its toxicity, its efficacy as an anesthetic, and its residues in tissues of treated fish. Similar studies were conducted on salmonids at the Fish Control Laboratories (Marking, 1966; Schoettger and Julin, 1966; and Walker and Schoettger, 1966a, 1966b). The history and development of M-222 as a fish anesthetic were reviewed by Bové (1962), the manufacturer (Sandoz Pharmaceuticals), Eisler and Backiel (1960), and Schoettger (1966).

The scope of the investigation on channel catfish was somewhat narrower than that of our earlier study on salmonids. We tested the influences of temperature or size of fish on toxicity and efficacy. The effects of water hardness on toxicity and of pH on efficacy were also measured. Determinations of MS-222 residues in catfish were confined to those in muscle because residues in blood, liver, and kidney of salmonids decreased with withdrawal time at a rate similar to that in muscle; because background substances of natural origin in the livers and kidneys of

Arnold M. Julin is now at the Fish Genetics Laboratory, Bureau of Sport Fisheries and Wildlife, Beulah, Wyo.

salmonids interfered with analyses of low residues; and because muscle is the principal edible tissue in catfish.

METHODS AND MATERIALS

The channel catfish used in the toxicity, efficacy, and residue experiments were obtained from the national fish hatcheries at Fairport and Guttenberg, Iowa, and from the Mississippi River (by the Iowa Conservation Department). They were held in well water at 12° C. and without feed for 2 days before placement in the anesthetic solutions. Twenty-four hours before an experiment, the fish were acclimated to temperature in reconstituted water prepared according to methods described by Lennon and Walker (1964).

TOXICITY

The static bioassays of MS-222 were conducted with 1.9-, 2.6-, and 3.5-inch fish according to the methods of Lennon and Walker (1964). They were tested in 5-gallon glass jars containing 15 liters of test solution, with aeration applied to solutions containing the 3.5-inch fish. Water temperatures were maintained at 12°, 17°, and 22° C. by placing the bioassay vessels in thermostatically controlled water baths.

Ten fish were exposed to each concentration, and 10 or 20 served as controls. Ten concentrations were selected to yield survival and mortality of 1.9- and 2.6-inch fish; 5 concentrations were selected for the 3.5-inch fish.

The data were analyzed statistically according to the method of Litchfield and Wilcoxon (1949) to define concentrations which produced 50 percent mortality (LC₅₀). In addition, the variances, slope functions, and 95 percent-confidence intervals were determined.

Different water qualities were obtained by varying the amounts of reconstituting salts added to deionized water (table 1).

Safety indexes (S.I.) were determined for the anesthesia of channel catfish with MS-222. We define the indexes as the margin between concentrations effective for anesthesia and those which cause mortality, expressed as a number obtained by dividing the lethal concentration (LC₅₀) by the effective concentration (EC₅₀). An effective level anesthetizes the catfish to loss of equilibrium. This stage of anesthesia is defined later in methods for measuring the efficacy of MS-222.

The S.I. values were calculated for 15-, 30-, and 60-minute exposures. These exposures were selected to give consistent results because shorter exposures required extremely high concentrations. In longer exposures, the anesthetized fish occasionally recovered in the test solutions.

The maximum safety indexes (M.S.I.) were calculated from the LC₁ and EC₉₉ values obtained by extrapolating the regressions used in determining LC₅₀ and EC₅₀ values. The maximum safety index is lower than the safety index and is biased in favor of greater safety.

Table 1.--Water qualities obtained with various amounts of reconstituting salts in deionized water

Water quality	Salts in mg./l.				Total hardness as CaCO ₃ (p.p.m.)	Total alkalinity as CaCO ₃ (p.p.m.)	pH
	NaHCO ₃	CaSO ₄	MgSO ₄	KCl			
Soft.....	12	7.5	7.5	0.75	10-13	10-15	6.4-6.8
Standard.....	48	30.0	30.0	3.00	40-48	30-35	7.2-7.6
Hard.....	192	120.0	120.0	12.00	160-180	110-120	7.6-8.0

EFFICACY

Channel catfish of 2 to 6 inches and 7 to 12 inches were used to determine the efficacy of MS-222. Fish less than 4 inches were tested in 15 liters of reconstituted water. Larger individuals were exposed in 45 liters. Since preliminary trials failed to show that variable loading had any effect on efficacy, the loading levels were approximately 10 g./l. for the smaller fish, and up to 40 g./l. for the larger fish.

The methods of evaluating efficacy of MS-222 against channel catfish were similar to those reported in our trials with salmonids. A series of concentrations of MS-222 were tested at a temperature of 12° C. and at pH 7.0. The levels giving reasonable anesthetization, holding and recovery times, and which produced minimum mortality were considered for further trials at 7°, 12°, 17°, 22°, and 27° C., and at pH 5.0, 7.0, and 8.5.

The criteria for assessing efficacy of MS-222 against channel catfish were changed slightly from those used for salmonids; therefore, a portion of the results which governed methodology are included in this section. The behavioral responses of catfish in deep anesthesia differed from those of trout. In catfish, at high concentrations of MS-222, we were unable to distinguish clearly the transition from total loss of equilibrium, stage II, into loss of reflex. The reflex response to constant pressure on the caudal fin or peduncle is frequently delayed for 1 to 10 seconds. Also, loss of reflex is not always distinct from medullary collapse. Opercular movements occasionally cease before reflex activity stops. A concentration of MS-222 was considered effective for rapid or moderately rapid anesthesia when it induced total loss of equilibrium, stage II, within 2 and 15 minutes respectively. This stage of anesthesia is more safely induced and maintained than loss of reflex, and the fish appear to be almost as easily handled. An effective concentration for sedation induces the response within 15 minutes and maintains it for 6 hours.

RESIDUES

Residues of MS-222 were measured in muscles of 6- to 13-inch channel catfish which had been treated during the efficacy trials. Groups, each composed of three individuals, were anesthetized to medullary collapse by 270 p.p.m. of MS-222 at 7°, 12°, 17°, 22°, and 27° C. In this series of tests, the drug was less effective at 7° and some fish tolerated an exposure exceeding 1 hour; at higher temperatures the exposures were approximately 5 to 10 minutes long.

Five or six groups of catfish were anesthetized at each temperature. One narcotized group was selected for each temperature for analyses of residues at 0-hour withdrawal. The remaining groups were placed in fresh water for recovery. Their muscles were analyzed 1, 3, 6, 9, and 24 hours after withdrawal. We also analyzed control fish at each temperature.

The residues of MS-222 in fish flesh were determined by the modified Bratton-Marshall method developed by Walker and Schoettger (1966a).

RESULTS

TOXICITY

Effects of size.--The toxicity of MS-222 to channel catfish is dependent, in part, on their size. The larger fish appear more resistant to the anesthetic than smaller individuals (table 2). The 24-hour LC₅₀ values for 1.9-, 2.6-, and 3.5-inch specimens are 58.0, 64.0, and 66.2 p.p.m., respectively. This size-toxicity relationship was also observed in the 48- and 96-hour bioassays.

Effects of temperature.--The toxicity of MS-222 to channel catfish is influenced by temperature. The data indicate a higher resistance of 1.9-inch fish at 17° than at 12° or 22° C. (table 2). The 24-hour LC₅₀ values for the latter two temperatures are similar, but

Table 2.--Toxicity of MS-222 to channel catfish at three temperatures

Temperature and lot	Average size		24 hours		48 hours		96 hours	
	Length (in.)	Weight (g.)	LC ₅₀ (p.p.m.)	95-percent confidence interval	LC ₅₀ (p.p.m.)	95-percent confidence interval	LC ₅₀ (p.p.m.)	95-percent confidence interval
At 12° C.:								
Lot 276.....	1.9	0.9	58.0	55.6-61.5	55.0	53.3-56.8	51.1	49.0-53.5
Lot 92.....	2.6	1.9	64.0	58.2-70.4	55.0	52.9-57.2	55.0	53.9-56.1
Lot 122.....	3.5	5.2	66.2	63.0-69.5	62.1	60.9-63.3	62.1	60.9-63.3
At 17° C.:								
Lot 276.....	1.9	0.9	60.5	58.1-62.9	60.0	58.3-61.7	60.0	58.3-61.7
At 22° C.:								
Lot 276.....	1.9	0.9	59.8	58.0-61.5	58.8	57.0-60.6	58.8	57.0-60.6

Table 3.--Toxicity of MS-222 in selected water qualities at 12° C.

Total hardness	Total alkalinity (p.p.m.)	24 hours			48 hours		96 hours	
		pH	LC ₅₀ (p.p.m.)	95-percent confidence interval	LC ₅₀ (p.p.m.)	95-percent confidence interval	LC ₅₀ (p.p.m.)	95-percent confidence interval
12 p.p.m...	15	6.6	64.0	58.7-69.8	60.0	57.1-63.0	50.0	46.7-53.5
46 p.p.m...	30	7.5	58.0	55.6-61.5	55.0	53.3-56.8	51.1	49.0-53.5
170 p.p.m...	114	7.8	54.0	51.0-57.4	54.0	51.0-57.4	54.0	52.6-55.5

the 96-hour LC₅₀ was lower at 12° than at 22° C. In general, the relative change in toxicity of the anesthetic with exposure is less at 17° and 22° than at 12° C.

Effect of water quality.--The toxicity of MS-222 to catfish was evaluated in relatively soft, medium, and hard water (table 3). The LC₅₀'s range from 50 to 64 p.p.m., and the drug is more toxic at 24 hours in hard water than in soft water. After 96 hours of exposure the drug appears more toxic in soft water. The toxicities of MS-222 to catfish in medium and hard waters are similar, except at 96 hours of exposure. Here, the LC₅₀ values for medium and soft water are much alike, and the 95-percent confidence limits overlap considerably.

The greatest change in toxicity of MS-222 with exposure occurred in tests in soft water. These trials also gave the most variable results as shown by the relative widths of the 95-percent confidence intervals (table 3).

Safety indexes.--The S.I. values in table 4 range from 2.4 to 3.0 and indicate that 15-minute exposures are safer than those lasting 30 or 60 minutes. The M.S.I. values, on the other hand, show greater safety with longer exposures. This is probably due to the large variance which is associated with the broad range of concentrations tolerated by channel catfish.

EFFICACY

The efficacy of MS-222 for inducing rapid and moderately rapid anesthesia and sedation in channel catfish appears to be unaltered by pH 5.0 to 8.5. These data were pooled, without differentiation, with those for size of fish and temperature.

Rapid anesthesia.--Concentrations of 140 to 270 p.p.m. are all equally effective for the rapid anesthesia of 7- to 12-inch catfish at 12° to 27° C. (table 5). Levels above 100 to 120 p.p.m. anesthetize 2- to 6-inch fish.

Table 4.--Safety indexes and maximum safety indexes for anesthesia of channel catfish with MS-222 at 12° C.

Exposure	Safety index			Maximum safety index		
	LC ₅₀ (p.p.m.)	EC ₅₀ (p.p.m.)	Index LC ₅₀ /EC ₅₀	LC ₁ (p.p.m.)	EC ₉₉ (p.p.m.)	Index LC ₁ /EC ₉₉
15 minutes.....	139.0	46.5	3.0	76.0	71.9	1.1
30 minutes.....	118.0	45.0	2.6	74.0	62.0	1.2
60 minutes.....	110.0	46.4	2.4	80.0	62.8	1.3

Table 5.--Concentrations of MS-222 producing rapid anesthesia in two sizes of channel catfish at five temperatures

Temperature and concentration	Size of fish (in.)	Lot No.	Fish in loss of equilibrium, stage II, within 2 minutes		Mean range of exposure times (min.)		Safe exposure index ¹	Recovery mean time range (min.)	Survival (percent)
			Number	Percent	First fish	Last fish			
At 7° C.:									
160-270 p.p.m.	2- 6	122	39/ 40	98	² 6	--	--	6-24	100
160-270 p.p.m.	7-12	114	24/ 30	80	² 6	--	--	6-13	100
200-270 p.p.m.	7-12	199 & 203	2/ 48	4	25	48	--	19-33	98
At 12° C.:									
80 p.p.m.	2- 6	122	0/ 40	0	² 30	--	--	2- 5	100
100 p.p.m.	2- 6	122	10/ 20	50	² 11	--	--	2- 9	100
120-260 p.p.m.	2- 6	122	331/345	95	² 7	--	--	7-18	97
270 p.p.m.	7-12	203	18/ 18	100	6	7	3.0	7-10	100
At 17° C.:									
80 p.p.m.	2- 6	122	0/ 10	0	² 20	--	--	8-15	100
100-220 p.p.m.	2- 6	122	90/ 90	100	² 6	--	--	3- 7	100
140-270 p.p.m.	7-12	114 & 203	63/ 80	79	8	11	4.0	4- 8	100
At 22° C.:									
270 p.p.m.	7-12	203	24/ 24	100	4	5	2.0	5- 7	100
At 27° C.:									
100-130 p.p.m.	7-12	203	12/ 25	48	7	9	--	3- 9	100
140-270 p.p.m.	7-12	203	53/ 55	96	4	5	2.0	5-10	80

¹ Index obtained by dividing the time for the first fish to reach medullary collapse by the time (2 min.) for all fish to reach loss of equilibrium, stage II.

² Fish removed from the anesthetic before all reach medullary collapse.

Catfish to tolerate exposures to MS-222 for approximately 6 to 11 minutes at 12° and 17°, and 4 to 5 minutes at 22° and 27° C. They usually recover in fresh water in less than 15 minutes; at 27°, however, 20 percent of the test fish died when they were not removed quickly enough to prevent overexposures.

The results of tests at 7° C. were variable (table 5). Concentrations of 160 to 270 p.p.m. were 80 to 98 percent effective on lots 114 and 122, but 200 to 270 p.p.m. were ineffective on lots 199 and 203. Although individuals in lots 114 and 122 were effectively anesthetized and tolerated exposure for about 6

minutes, lots 199 and 203 required 3 to 5 minutes for anesthesia, and some individuals tolerated exposures for more than an hour.

Fish in lot 199 were used for residue analyses, and the numbers remaining were insufficient for comparative tests to determine whether they were also more resistant than lot 114 at higher temperatures. The sensitivities of catfish in lot 203 were similar to those in lot 199 at 7° and those in lot 114 at 17° C., and the results were combined at the respective temperatures. The purpose in comparing sensitivities of the different lots is to point out that there was some variable in the tests at 7° which increased the resistance of catfish to MS-222 in one instance but not in another. Analysis of the sources of the fish and their general conditions indicated that these were probably not causes of the inconsistencies. On the other

hand, fish in lot 114 were tested in the fall, and those in lots 199 and 203 in the spring. It is conceivable that the natural acclimation of the latter fish to low temperatures during the winter may have contributed to their resistance to MS-222 at 7° C.

We were unable to calculate safe exposure indexes for all of the trials reported in table 5. In most instances the fish were placed in fresh water before they all entered medullary collapse. The indexes in the remaining trials range from 2 to 4. They indicate that catfish can be safely exposed to MS-222 for about 2 to 4 times longer than required to induce loss of equilibrium, stage II.

Moderately rapid anesthesia.--Seventy p.p.m. of MS-222 induce loss of equilibrium in channel catfish within 15 minutes (table 6). They tolerate exposure to this concentration

Table 6.--Concentrations of MS-222 producing moderately rapid anesthesia in two sizes of channel catfish at four temperatures

Concentration, temperature and size of fish	Lot No.	Fish in loss of equilibrium, stage II, within 15 minutes		Exposure time (min.)	Recovery	
		Number	Percent		Mean time range (min.)	Survival (percent)
At 70 p.p.m.:						
At 7° C.:						
2- 6 in.....	122	10/10	100	30	2- 4	100
7-12 in.....	114	6/ 6	100	30	2- 6	100
At 12° C.:						
2- 6 in.....	122	70/70	100	30	3- 5	100
7-12 in.....	114	33/35	94	30	2-10	100
At 17° C.:						
2- 6 in.....	122	10/10	100	30	1- 2	100
At 60 p.p.m.:						
At 27° C.:						
7-12 in.....	203	4/10	40	¹ 24	--	100
At 70 p.p.m.:						
At 27° C.:						
7-12 in.....	203	8/8	100	180	1- 2	75

¹ Hours

for at least 30 minutes and many can be exposed longer. In one test, at 27° C., the majority of the fish survived at 3-hour exposure.

Water temperature and size of fish appear to have no influence on the efficacy of a concentration inducing a moderate rate of anesthesia. The fish recover faster, however, at 22° and 27° than at 7°, 12°, or 17°. The sensitivities of the different lots of test fish at various temperatures were only partially compared.

A moderate rate of anesthesia appears most applicable to handling operations such as measuring and weighing, or when large numbers of individuals are involved. A level of 70 p.p.m. may also be useful for long-term surgical operations, provided sufficient time is allowed for reflex responses to decline.

Sedation.--Channel catfish are sedated in 20 p.p.m. of MS-222 at 7°, 12°, and 17° C. (table 7). This level was not effective at 27° C.,

and the concentration was increased to 40 p.p.m. to maintain sedation for 6 hours. These data suggest that sedating concentrations of MS-222 are metabolized or deactivated at higher temperatures. This finding is supported by the results of McFarland (1959 and 1960), Schoettger and Julin (1966), and our toxicity trials with catfish.

RESIDUES

Residues of MS-222 occur in the muscles of anesthetized channel catfish and appear to vary with temperature. Seventeen to 147.2 p.p.m. of the drug, including background aromatic amines, were measured in fish narcotized to medullary collapse in 270 p.p.m. of MS-222 (table 8). The mean concentrations of residue at 0-hour withdrawal are: 31.3 p.p.m. at 7° C., 69.9 p.p.m. at 12°, 125.5 p.p.m. at 17°, 97.5 p.p.m. at 22°, and 65.7 p.p.m. at 27°. The levels in fish treated at 12°, 17°, 22°, and 27° are two to four times greater than in those exposed at 7° C.

Table 7.--Concentrations of MS-222 producing sedation in two sizes of channel catfish at four temperatures

Concentration, temperature, and size of fish	Lot No.	Fish in sedation at--				Behavior ¹ of fish not in sedation at--	
		15 minutes		6 hours			
		Number	Percent	Number	Percent	15 min.	6 hrs.
At 20 p.p.m:							
At 7°C.:							
2-6 in.....	122	10/ 10	100	10/ 10	100	--	--
7-12 in.....	114	6/ 6	100	6/ 6	100	--	--
At 12° C.:							
2- 6 in.....	122	138/140	99	132/140	94	<>	>
At 17°C:							
2- 6 in.....	122	50/ 50	100	50/ 50	100	--	--
7-12 in.....	114	6/ 6	100	6/ 6	100	--	--
At 27°C.:							
7-12 in.....	203	12/ 12	100	0/ 12	0	--	<
At 30 p.p.m:							
At 27°C.:							
7-12 in.....	203	13/ 13	100	8/ 13	62	--	<
At 40 p.p.m.:							
At 27°C.:							
7-12 in.....	203	6/ 6	100	² 6/ 6	100	--	--

¹ > = deeper anesthesia; < = similar to controls.

² Fish in sedation at 3 and 19 hours.

Table 8.--Residues of MS-222 including background in muscle of channel catfish for various withdrawal times at selected temperatures

Temperature and withdrawal time	Number of fish	Mean size		Residues in p.p.m.		Standard error	95-percent confidence interval
		Length (in.)	Weight (g.)	Mean	Range		
At 7° C.:							
0 hour.....	3	12.13	199.7	31.30	17.0- 38.5	7.26	---
1 hour.....	3	12.53	223.0	26.70	22.5- 34.9	4.10	8.58-44.34
6 hours.....	3	12.53	214.3	8.83	7.6- 11.2	1.18	1.08-16.58
9 hours.....	3	12.03	223.3	5.07	4.0- 5.8	0.55	2.70- 7.44
24 hours.....	3	12.77	248.0	4.53	2.5- 7.6	0.91	0.61- 8.45
At 12° C.:							
0 hour.....	3	10.57	140.7	69.93	56.8- 77.2	6.58	---
1 hour.....	3	7.87	47.3	6.57	4.9- 9.3	1.40	0.55-12.59
3 hours.....	3	8.27	61.7	1.20	0.5- 1.2	0.47	0.00- 3.22
6 hours.....	3	7.73	45.0	1.10	0.8- 1.3	0.15	0.45- 1.75
9 hours.....	3	8.07	51.7	0.83	0.6- 1.0	0.18	0.06- 1.60
24 hours.....	3	7.87	49.3	0.90	0.8- 1.0	0.06	0.64- 1.16
At 17° C.:							
0 hour.....	3	9.00	77.0	125.53	88.6-147.2	18.55	---
1 hour.....	3	8.83	67.7	6.57	4.8- 8.7	1.15	1.62-11.52
3 hours.....	3	9.20	80.7	1.40	1.3- 1.5	0.05	1.19- 1.62
9 hours.....	3	9.33	84.3	2.23	1.7- 2.8	0.32	0.85- 3.61
24 hours.....	3	9.00	78.3	1.00	0.7- 1.4	0.21	0.10- 1.90
At 22° C.:							
0 hour.....	3	8.90	67.3	97.47	87.6-105.4	5.23	---
1 hour.....	3	7.70	45.7	6.57	4.5- 9.3	1.43	0.42-12.72
3 hours.....	3	8.83	65.3	1.47	1.2- 1.9	0.22	0.52- 2.42
6 hours.....	3	7.53	41.0	1.20	0.9- 1.7	0.25	0.12- 2.28
9 hours.....	3	8.17	56.7	1.27	0.8- 1.8	0.29	0.02- 2.52
24 hours.....	3	8.47	57.3	0.60	0.5- 0.7	0.06	0.34- 0.86
At 27° C.:							
0 hour.....	3	8.80	71.7	65.67	37.2- 80.0	14.23	---
1 hour.....	3	9.13	71.0	4.00	2.0- 6.4	1.29	0.00- 9.55
3 hours.....	3	9.23	80.0	4.23	1.9- 6.8	0.59	1.67- 6.77
6 hours.....	3	8.83	64.3	1.47	0.9- 1.9	0.30	0.18- 2.76
9 hours.....	3	8.83	64.0	1.27	0.8- 1.6	0.24	0.24- 2.30
24 hours.....	3	8.77	62.0	0.77	0.6- 1.0	0.12	0.25- 1.29

As in salmonids, a background of aromatic amines is also present in the muscles of un-anesthetized catfish. The background in catfish ranges from 0.5 to 3.6 p.p.m. (table 9). Both the upper and the lower limits of the range occur in samples from fish (lot 199, table 5) maintained at 7°. The mean background at this temperature is 1.7 p.p.m. with a standard error of 0.96. The 95-percent confidence interval around the means is 0 to 5.33 p.p.m. The mean concentrations in flesh at other temperatures range from 0.8 to 1.5 p.p.m. with standard errors from 0.05 to 0.18.

Their 95-percent confidence intervals overlap between 0.10 and 1.72 p.p.m.

The residues of MS-222 in muscles of catfish decline approximately 90 to 95 percent after 1 hour of withdrawal at 12°, 17°, 22°, and 27° C. (table 8). They decrease further after 6 to 24 hours of withdrawal and are within the 95-percent confidence intervals around the mean backgrounds of controls. The residues in catfish at 7° decline more slowly than at higher temperatures, but are within the background

Table 9.--Background residues of aromatic amines in muscle of channel catfish used as controls in the study of MS-222

Temperature	Number of fish	Mean size		Concentration in p.p.m.		Standard error	95-percent confidence interval
		Length (in.)	Weight (g.)	Mean	Range		
7° C.....	3	12.20	220.7	1.70	0.5-3.6	0.96	0.00-5.83
12° C.....	5	9.80	108.2	0.80	0.6-1.0	0.07	0.61-0.99
17° C.....	3	9.20	80.7	1.07	0.9-1.3	0.12	0.55-1.59
22° C.....	3	9.43	80.7	1.50	1.4-1.5	0.05	1.28-1.72
27° C.....	3	8.37	51.7	0.87	0.6-1.2	0.18	0.10-1.65

Table 10.--Acetylated aromatic amines in muscle of channel catfish for various withdrawal times at 7° C.

Withdrawal time	Number of fish	Mean size		Residues in p.p.m.		Standard error	95-percent confidence interval	Percent of total aromatic amines
		Length (in.)	Weight (g.)	Mean	Range			
0 hour.....	3	12.1	199.7	1.07	0.0-2.4	0.71	---	2.8
1 hour.....	3	12.5	223.0	3.76	2.4-5.1	0.78	0.40-7.12	12.4
6 hours.....	3	12.5	214.3	5.50	4.8-6.2	0.40	3.76-7.24	38.4
9 hours.....	3	12.0	223.3	6.00	5.3-7.0	0.56	3.61-8.29	54.2
24 hours.....	3	12.8	248.0	3.83	2.4-4.9	0.75	0.62-7.04	47.9

confidence interval for this temperature after 9 to 24 hours of withdrawal.

DISCUSSION

TOXICITY

The channel catfish is one of the species more resistant to MS-222. The 24- to 96-hour LC₅₀'s range from 66.2 to 51.1 p.p.m. Marking (1966b) reported LC₅₀'s for similar exposures of 52.2 to 31.0 p.p.m. for various salmonids and 61.5 to 39.4 p.p.m. for northern pike, bluegill, largemouth bass, and walleye. This greater resistance of catfish to certain chemicals has been noted in other investigations at this laboratory (Walker et al., 1964; Marking, 1966a). Also, large catfish are more resistant to the drug than small ones, and this finding agrees with the size-sensitivity relation between other species and MS-222 noted by Marking (1966b).

Catfish are somewhat more resistant to MS-222 at 17° than at 12° or 22° C. Marking (1966b) made a similar observation on the toxicity of the anesthetic to bluegill. Whether the resistance of 1.9-inch catfish at 17° C. is

Channel catfish which had been anesthetized at 7° were also analyzed to detect possible acetylated derivatives of MS-222 (table 10). The presence of these derivatives may indicate a mechanism within the fish for deactivation of the drug. The acetylated fraction is obtained by subtracting the free MS-222 and background concentrations (table 8) from the total aromatic amines measured in the sample after acid hydrolysis. At 0-hour withdrawal the muscles contain an average of 1.07 p.p.m. of acetylated aromatic amines, or 2.8 percent of the total aromatic amines. When the fish are transferred to fresh water, the concentrations of acetylated substances increase to a mean high of 6 p.p.m. after 9 hours of withdrawal, and then decline. Within this period, free residues decrease so that the relative amount of acetylated amines increase to 54.2 percent. The concentrations of both fractions decline at 24 hours of withdrawal, but their proportions are similar.

significant seems doubtful. The confidence interval for the 24-hour LC₅₀ at this temperature includes the LC₅₀ for 1.9-inch fish at 22° and almost includes the value at 12°.

The interaction of temperature and exposure seems to have a greater effect on toxicity of MS-222 than temperature alone. The fact that the drug increases in toxicity with time at 12°, but not significantly at 17° and 22° suggests that it degrades at the higher temperatures.

We found that catfish are relatively more resistant when the anesthetic is dissolved in soft water. The resistance declines with exposure, and after 96 hours the soft water solutions are slightly more toxic. Schoettger and Julin (1966) reported that MS-222 is less effective against rainbow trout in very soft water. They suggested that the rate of absorption and deactivation of the drug may increase along with higher metabolic rates of trout in soft water. The decreased resistance of catfish with time, however, may reflect the effects of osmotic stress.

The safety indexes for anesthesia of channel catfish with MS-222 show that the lethal concentrations of drug are approximately 2.5 to 3.0 times greater than the levels which induce narcosis. In actual practice this method of evaluating safety of fish anesthetics is not entirely applicable. The fish may be anesthetized at similar or slightly higher concentrations than those used to calculate safety indexes, but exposure may determine lethality. For this reason some indexes based on safe exposure were calculated in the efficacy experiments.

EFFICACY

MS-222 is effective and safe for the rapid and moderately rapid anesthesia of channel catfish. The concentrations for the former rate of narcotization, 100 to 270 p.p.m., exceed the 80 to 135 p.p.m. which were effective for salmonids (Schoettger and Julin, 1966) and suggest that catfish tolerate a much broader range of concentrations. The levels for moderate rates in salmonids and catfish are simi-

lar. The low end of the range of concentrations inducing rapid anesthesia in catfish probably represents a threshold, or minimum effective level, since concentrations up to 270 p.p.m. are essentially no more effective. Concentrations within this range have been used to anesthetize channel catfish for sexing and physiological investigations (Crawford and Hulsey, 1963; Larsen, 1964; Moss and Scott, 1964).

The inconsistent efficacy of MS-222 on three lots of fish at 7° C. may reflect differential sensitivities of the lots. The differences may be related to prior acclimation to environmental temperatures. Residues of MS-22 were later measured in muscles of fish in a resistant lot and, as discussed elsewhere, the residues differ markedly from those in fish treated at higher temperatures.

In general, temperature has little influence on the efficacy of MS-222 against catfish, but they tolerate less exposure at 22° and 27° than at 7°, 12°, or 17° C. It was necessary to increase the concentrations of the drug for salmonids at lower temperatures, but a similar influence of temperature on the resistance of catfish may have been masked by the broad range of effective concentrations.

Although channel catfish appear to be more resistant to MS-222 than salmonids, the durations of exposure tolerated by both are similar. The behavioral responses of the catfish must be observed, however, to minimize the risk of overexposures. Medullary collapse occurs almost simultaneously with loss of reflex, and therefore, anesthesia to the latter stage may be unsafe and unnecessary. The delayed reflex response which occurs late in loss of equilibrium does not usually preclude the handling of fish. The cessation of opercular activity in catfish, as in trout, is a good criterion for transferring them to fresh water, although many individuals may recover completely from brief exposures beyond this point.

Sedation can be induced and maintained in channel catfish by MS-222 at 20 to 40 p.p.m., depending on temperature. Schoettger and Julin (1966) cited contradictory reports on

the benefits of sedating various species of fish with the drug for transport. We are unable to locate published reports on the use of MS-222 for transport of channel catfish.

RESIDUES

The mean residues of MS-222 in the flesh of anesthetized channel catfish at 12° and 17° C. are approximately two to eight times as great as mean residues measured by Walker and Schoettger (1966a) in salmonids at the same temperatures. The mean values at 7° are similar. This indicates that relatively more MS-222 is taken up by salmonids at colder temperatures than at warmer temperatures, whereas the reverse applied to catfish. One explanation for the differential deposition of MS-222 in salmonids and catfish may be the influence of temperature on the efficacy of MS-222 in salmonids, and a relative lack of its effect in catfish. The concentrations of MS-222 were adjusted upward to maintain efficacy against salmonids at lower temperatures, but only one level, two to three times that for salmonids, was used to anesthetize catfish in the residue experiments. On the other hand, the differential may represent the influence of theoretical temperature optimums or acclimation on drug metabolism.

Residues of MS-222 in salmonids and channel catfish decline rapidly during the first hour of withdrawal, except those in catfish at 7°. The latter are initially low and decrease gradually. The acclimation of these individuals to winter temperatures may have contributed to their low uptake and turnover of MS-222, and to their resistance to anesthetization at 7°. The background of aromatic amines in untreated catfish is slightly higher at 7° and suggests a temperature-related reduction in metabolism of these substances. Residues of the drug, at all temperatures, cannot be distinguished from the background in controls when the fish are held in fresh water for 9 to 24 hours after anesthesia.

Interestingly, the mean concentrations of residues at 0-hour withdrawal form a parabolic curve when plotted against temperature, and peak at 17° C. We have no explanation for this, unless it is caused by a temperature-

dependent, uptake-and-metabolism relation. The pattern does not persist after withdrawal.

The acetylation of MS-222 may be an effective mechanism for its deactivation in catfish. The concentrations of acetylated derivatives in muscles of fish treated at 7° increase during withdrawal times up to 9 hours, and as the amount of free MS-222 decreases. They decline after 24 hours of withdrawal, when the concentration of the free fraction is lowest. The inverse change in concentrations of free and acetylated derivatives during withdrawal suggests metabolism of MS-222. The absolute amounts of acetylated substances are relatively small, however, and may simply reflect the turnover of natural aromatic amines.

SUMMARY

MS-222 was tested against channel catfish to determine its toxicity, efficacy, and residues in muscle. Several size groups of fish were exposed to solutions with various temperatures, pH, and water qualities.

The LC₅₀ values of MS-222 for catfish range from 66.2 to 51.1 p.p.m. in 24- to 96-hour bioassays. The variations in toxicity are associated with exposure, size of fish, temperature, and water quality. Channel catfish are more resistant to the drug than other species we have tested. The safety indexes for anesthesia of catfish decrease from 3.0 to 2.4 with exposures of 15 to 60 minutes.

Concentrations of drug above 100 to 140 p.p.m. are effective for inducing rapid loss of equilibrium in catfish within 2 minutes. They tolerate exposure for 4 to 11 minutes. A level of 70 p.p.m. produces moderately rapid anesthesia, and 20 to 40 p.p.m. are effective for sedation. The fish tolerate concentrations for moderately rapid anesthesia for at least 30 minutes. They can be maintained in sedation for 6 hours by 20 to 40 p.p.m. The efficacy of MS-222 is not influenced greatly by temperature, pH, or size of fish. At 22° or 27° C., the fish tolerate less exposure to high drug concentrations, and at 27° the sedation concentration has to be increased to compensate for an apparent degradation of MS-222.

The deep stages of MS-222-induced narcosis, beyond loss of equilibrium, appear less distinct in catfish than in salmonids. The behavior of the fish must be observed to minimize the risk of overexposures, and cessation of opercular activity is a good criterion for transferring them to fresh water.

Residues of MS-222 occur in the muscles of anesthetized catfish. They vary, according to temperature, from 17.0 to 147.2 p.p.m. including background aromatic amines. The background, in control fish, ranges from 0.5 to 3.6 p.p.m. The lowest concentrations of residue at 0-hour withdrawal occur in fish treated at 7° C., but after 1 hour of withdrawal from the drug the levels in individuals exposed at this temperature are highest. The residues in muscles after 9 to 24 hours of withdrawal at all temperatures decline to within the statistical variations in the background concentrations.

The concentrations of acetylated aromatic amines in muscles of catfish anesthetized at 7° C. increase as MS-222 residues decrease during 9 hours of withdrawal. The inverse relation suggests metabolism of MS-222 by acetylation, but the small amounts of acetylated derivatives may be aromatic amines of natural origin.

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19. Toxicity of Bayer 73 to Fish

20. Toxicity of Dimethyl Sulfoxide (DMSO) to Fish

21. Labor-Saving Devices for Bioassay Laboratories



**United States Department of the Interior
Fish and Wildlife Service
Bureau of Sport Fisheries and Wildlife**

INVESTIGATIONS IN FISH CONTROL

Investigations in Fish Control, published by the Bureau of Sport Fisheries and Wildlife, include reports on the results of work at the Bureau's Fish Control Laboratories at La Crosse, Wis., and Warm Springs, Ga., and reports of other studies related to that work. Though each report is regarded as a separate publication, several may be issued under a single cover, for economy.

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TOXICITY OF 22 THERAPEUTIC COMPOUNDS TO SIX FISHES

By Wayne A. Willford, Chemist
Fish Control Laboratory, La Crosse, Wis.

ABSTRACT.--Of 22 therapeutic chemicals (18 parasiticides and 4 oral bacteriostats) tested by bioassays, 16 were toxic to fish and 6 were not. Tests were in 24- and 48-hour static bioassays on rainbow, brown, brook, and lake trout and bluegills at 12° C. and channel catfish at 17° C. The 16 toxic chemicals, in descending order, were malachite green, Trolene, CoRal, Tiguvon, Roccal, P.M.A., Acriflavine, amopyroquin dihydrochloride, merthiolate, methylene blue, Neguvon, Ruelene, TV-1096, nickel sulfate, formalin, and quinacrine hydrochloride; the 6 that did not appear to be toxic were erythromycin thiocyanate, quinine hydrochloride, Flagyl, sulfamerazine, sulfamethazine, and sulfisoxazole.

An objective of the Fish Control Laboratories is to develop chemical tools to prevent and control fish diseases. Although efficacious concentrations of many drugs have been determined, a thorough examination of their toxicity has not been reported. Prior to clearance of drugs, the Food and Drug Administration requires data on their toxicity. The purpose of this study was to define the toxicity of 22 therapeutic chemicals to six species of fish before further research is undertaken on their efficacy and residues.

Eighteen parasiticides of known or possible value as external treatments for fish were selected for investigation upon recommendations by other investigators. Four oral bacteriostats were tested to determine whether any toxicity to fish would result through leaching, or excretion, of the compounds into water.

MATERIALS AND METHODS

Six species of fish were obtained from various fish hatcheries (table 1). All were quarantined for 10 days, and those judged acceptable for bioassays were acclimated to conditions of the tests.

The static bioassays were made in facilities described by Lennon and Walker (1964). We used 5-gallon glass jars which contained 15 liters of reconstituted, deionized water at total hardness of 42 p.p.m., and a maximum of 1 gram of fish per liter of water. Each test included 10 concentrations of a chemical. Ten fish were exposed to each concentration, and 20 fish served as controls.

The 22 therapeutic compounds were tested at 12° C. against five species of fish at La Crosse, Wis. (table 2). Tests against channel catfish at 17° were made at the Southeastern Fish Control Laboratory, Warm Springs, Ga.

A concentrated stock solution of each compound, using acetone or deionized water or both as solvents, was usually prepared for addition to the test vessels immediately before each test. When solubility of the compound prevented preparation of concentrated stocks, the compound was added directly and allowed to dissolve in the test vessel.

Observations on survival and mortality were recorded at 24 and 48 hours. The data were then analyzed by plotting concentration against mortality on logarithmic normal

TABLE 1.--Fishes used in toxicity trials

Species	Lot	Average length (inches)	Average weight (grams)	Grading date	Source
Rainbow trout, <i>Salmo gairdneri</i>	159	1.5	0.5	1-21-65	National Fish Hatchery, Manchester, Iowa
Do.....	159	1.8	0.9	2-15-65	
Brown trout, <i>Salmo trutta</i>	177	1.7	0.8	3-16-65	National Fish Hatchery, Lake Mills, Wis.
Do.....	177	1.9	1.2	4- 1-65	
Brook trout, <i>Salvelinus fontinalis</i>	161	1.5	0.4	1-21-65	State Fish Hatchery, St. Croix Falls, Wis.
Do.....	161	1.6	0.6	2-15-65	
Lake trout, <i>Salvelinus namaycush</i>	78	4.0	2.5	8-14-64	National Fish Hatchery, Jordan River, Mich.
Do.....	78	4.0	2.8	8-28-64	
Do.....	78	4.1	3.2	10- 7-64	
Channel catfish, <i>Ictalurus punctatus</i> ...	W-70	2.1	1.2	7-21-65	National Fish Hatchery, Marion, Ala.
Do.....	W-74	2.2	1.5	8- 4-65	
Bluegill, <i>Lepomis macrochirus</i>	115	1.6	0.8	11- 5-64	National Fish Hatchery, Lake Mills, Wis.
Do.....	131	1.4	0.7	11-17-64	
Do.....	131	1.7	1.1	12- 1-64	

TABLE 2.--Common names and active ingredients of compounds tested

Common name	Grade or formulation	Active ingredient
Acriflavine (neutral).....	technical.....	3,6-diamino-10-methyl acridinium chloride and 3,6-diaminoacridine
Amopyroquin dihydrochloride.....	technical.....	4-(7-chloro-4-quinolylamino)- α -1-pyrrolidyl- α -resol dihydrochloride
CoRal.....	technical.....	0,0-diethyl 0-3-chloro-4-methyl-2-oxo-2H-1-benzopyran-7-yl-phosphorothioate
Erythromycin thiocyanate.....	800 mcg/mg.....	erythromycin thiocyanate
Flagyl.....	technical.....	1-(2-hydroxyethyl)-2-methyl-5-nitroimidazole
Formalin.....	U.S.P.....	37-percent formaldehyde gas in water
Malachite green.....	technical.....	bis-(p-dimethylaminophenyl) phenylmethane treated with HCL
Merthiolate.....	technical.....	sodium ethylmercurithiosalicylate
Methylene blue.....	technical.....	3,7-bis(dimethylamino) phenazathionium chloride
Neguvon.....	80-percent soluble powder.....	0,0-dimethyl 2,2,2-trichloro-1-hydroxyethyl phosphonate
Nickel sulfate.....	analytical reagent.....	NiSO ₄ · 6H ₂ O
P.M.A.....	technical.....	pyridylmercuric acetate
Quinacrine hydrochloride (Atabrine).....	technical.....	3-chloro-7-methoxy-9- (1-methyl-4-diethylaminobutylamino) acridine dihydrochloride
Quinine hydrochloride.....	technical.....	quinine hydrochloride
Roccal.....	50-percent concentrate.....	alkyl dimethylbenzylammonium chlorides
Ruelene.....	227 mg/cc.....	4-tert-butyl-2-chlorophenyl methyl methylphosphoramidate
Sulfamerazine.....	U.S.P.....	N ¹ -(4-methyl-2-pyrimidyl) sulfanilamide
Sulfamethazine.....	U.S.P.....	N ¹ -(4,6-dimethyl-2-pyrimidinyl) sulfanilamide
Sulfisoxazole.....	U.S.P.....	N ¹ -(3,4-dimethyl-5-isoxazolyl) sulfanilamide
Tiguvon.....	300 mg/cc.....	0,0-dimethyl 0-[4-(methylthio)-m-tolyl] phosphorothioate
Trolene.....	technical.....	0,0-dimethyl 0-2,4,5-trichlorophenyl phosphorothioate
TV-1096 (Parke, Davis & Company).....	technical.....	Lg-threo-2-(5-nitro-2-furyl)-5-(p-nitrophenyl)-2-oxazoline-4-methanol

(probability) graph paper to define the concentration that produced 50-percent mortality (LC₅₀) as described by Litchfield and Wilcoxon (1949). Variance and the 95-percent confidence interval (C.I.) were also determined.

Most of the compounds tested were technical or U.S.P. materials, and the rest were formulated materials. To eliminate confusion, all results are reported in terms of p.p.m. of

total material (formulated or technical) instead of active ingredient.

RESULTS

Of the 22 compounds, 16 were toxic to the six species of fish, and the LC₅₀ values were determined (tables 3 to 8).

The most toxic compound, malachite green, is relatively uniform in toxicity to the six

TABLE 3.--Toxicity of 16 compounds to rainbow trout at 12° C.

Compound	At 24 hours		At 48 hours	
	LC ₅₀ (p.p.m.)	95-percent C.I.	LC ₅₀ (p.p.m.)	95-percent C.I.
Acriflavine.....	40.1	26.2-34.6	19.9	17.0-23.3
Amopyroquin.....	42.0	33.5-50.8	39.3	33.3-37.4
CoRal.....	0.92	0.28-2.96	0.55	0.51-0.59
Formalin.....	20	182-236	168	154-183
Malachite green.....	0.30	0.36-0.69	0.39	0.33-0.46
Merthiolate.....	80.5	53.5-68.4	21.2	18.6-24.2
Methylene blue.....	24.2	20.5-30.5	16.0	13.8-18.7
Neguvon.....	32.3	29.3-36.1	12.2	10.6-14.0
Nickel sulfate.....	330	102-339	160	150-171
P.M.A.....	5.00	4.35-5.75	3.75	3.02-4.65
Quinacrine HCL.....	--	--	172	159-186
Roccal.....	3.24	2.92-3.60	2.57	2.16-3.06
Ruelene.....	35.0	31.5-38.8	32.0	30.5-33.6
Tiguvon.....	5.30	4.82-5.83	4.35	3.62-5.22
Trolene.....	1.17	0.89-1.53	0.74	0.64-0.86
TV-1096.....	24.2	22.8-25.7	16.1	14.9-17.4

TABLE 6.--Toxicity of 16 compounds to lake trout at 12° C.

Compound	At 24 hours		At 48 hours	
	LC ₅₀ (p.p.m.)	95-percent C.I.	LC ₅₀ (p.p.m.)	95-percent C.I.
Acriflavine.....	37.5	34.7-40.5	28.0	24.4-32.2
Amopyroquin.....	15.5	12.1-19.8	14.0	10.8-18.1
CoRal.....	6.80	4.00-11.56	4.00	1.29-12.80
Formalin.....	220	200-242	167	160-174
Malachite green.....	0.57	0.49-0.66	0.40	0.33-0.49
Merthiolate.....	13.0	9.6-17.6	2.13	1.06-4.26
Methylene blue.....	35.0	29.4-41.6	34.0	29.3-39.4
Neguvon.....	41.0	38.7-46.5	9.00	7.20-11.25
Nickel sulfate.....	170	139-209	75.0	55.6-101.2
P.M.A.....	12.5	11.8-13.2	7.00	6.33-9.12
Quinacrine HCL.....	28.0	18.8-42.0	21.0	12.4-35.7
Roccal.....	2.70	2.41-3.02	1.95	1.68-2.26
Ruelene.....	27.0	25.0-29.2	27.0	23.9-30.5
Tiguvon.....	6.50	6.08-6.96	5.30	4.91-5.72
Trolene.....	0.73	0.62-0.86	0.62	0.53-0.72
TV-1096.....	32.0	28.6-35.8	16.5	13.2-20.6

TABLE 4.--Toxicity of 16 compounds to brown trout at 12° C.

Compound	At 24 hours		At 48 hours	
	LC ₅₀ (p.p.m.)	95-percent C.I.	LC ₅₀ (p.p.m.)	95-percent C.I.
Acriflavine.....	40.0	36.4-44.0	27.0	25.0-29.2
Amopyroquin.....	42.0	37.5-47.0	36.0	33.3-38.9
CoRal.....	0.92	0.84-1.00	0.73	0.62-0.86
Formalin.....	325	304-348	185	165-208
Malachite green.....	0.45	0.42-0.49	0.34	0.30-0.38
Merthiolate.....	110	75-160	54.0	47.8-61.0
Methylene blue.....	54.0	46.2-63.2	32.8	28.8-37.4
Neguvon.....	54.0	48.2-60.5	16.5	11.8-23.1
Nickel sulfate.....	460	345-464	270	241-302
P.M.A.....	9.30	8.30-10.42	6.22	5.71-6.78
Quinacrine HCL.....	390	361-421	230	184-288
Roccal.....	2.95	2.46-3.54	2.05	1.74-2.42
Ruelene.....	26.2	24.7-27.8	25.7	24.2-27.2
Tiguvon.....	4.50	4.09-4.95	3.62	2.78-4.71
Trolene.....	0.53	0.38-0.74	0.39	0.30-0.51
TV-1096.....	--	--	--	--

TABLE 7.--Toxicity of 16 compounds to channel catfish at 17° C.

Compound	At 24 hours		At 48 hours	
	LC ₅₀ (p.p.m.)	95-percent C.I.	LC ₅₀ (p.p.m.)	95-percent C.I.
Acriflavine.....	43.5	39.9-47.4	33.2	31.0-35.5
Amopyroquin.....	19.8	17.7-22.2	12.5	11.8-13.2
CoRal.....	6.80	5.81-7.96	--	--
Formalin.....	137	129-145	96.0	90.6-101.8
Malachite green.....	0.21	0.17-0.27	0.20	0.16-0.26
Merthiolate.....	7.50	6.41-8.78	5.65	4.79-6.67
Methylene blue.....	120	110-131	104	93-116
Neguvon.....	80.0	72.7-88.0	32.0	24.8-41.3
Nickel sulfate.....	368	334-405	165	129-211
P.M.A.....	3.22	2.66-3.90	2.89	2.60-3.21
Quinacrine HCL.....	198	169-232	70.0	59.3-82.6
Roccal.....	1.28	1.16-1.41	1.12	1.03-1.22
Ruelene.....	39.5	37.6-41.5	34.8	32.5-37.2
Tiguvon.....	5.90	4.50-7.73	5.90	4.50-7.73
Trolene.....	1.76	1.54-2.01	1.26	1.09-1.46
TV-1096.....	27.0	24.8-29.4	20.3	19.3-21.3

TABLE 5.--Toxicity of 16 compounds to brook trout at 12° C.

Compound	At 24 hours		At 48 hours	
	LC ₅₀ (p.p.m.)	95-percent C.I.	LC ₅₀ (p.p.m.)	95-percent C.I.
Acriflavine.....	48.0	43.2-53.3	14.8	14.0-15.7
Amopyroquin.....	52.0	44.8-60.3	40.0	38.1-42.0
CoRal.....	1.06	0.87-1.29	0.80	0.70-0.91
Formalin.....	196	187-206	157	143-173
Malachite green.....	0.30	0.22-0.40	0.26	0.22-0.31
Merthiolate.....	89.5	85.2-94.0	74.5	71.0-78.2
Methylene blue.....	49.8	41.2-60.3	22.9	17.2-30.5
Neguvon.....	34.0	23.4-49.3	16.8	14.1-20.0
Nickel sulfate.....	--	--	242	224-261
P.M.A.....	15.5	12.9-18.6	10.7	9.8-11.7
Quinacrine HCL.....	--	--	230	177-299
Roccal.....	4.13	3.79-4.50	3.40	3.09-3.74
Ruelene.....	36.8	34.4-39.4	35.0	31.5-38.8
Tiguvon.....	6.15	5.21-7.26	5.50	5.14-5.88
Trolene.....	0.59	0.44-0.78	0.39	0.26-0.59
TV-1096.....	29.3	26.4-32.5	19.0	16.8-21.5

TABLE 8.--Toxicity of 16 compounds to bluegills at 12° C.

Compound	At 24 hours		At 48 hours	
	LC ₅₀ (p.p.m.)	95-percent C.I.	LC ₅₀ (p.p.m.)	95-percent C.I.
Acriflavine.....	18.0	16.8-19.3	13.5	12.6-14.4
Amopyroquin.....	33.0	23.6-42.2	18.5	16.7-20.5
CoRal.....	10.5	8.1-13.6	8.00	6.11-10.48
Formalin.....	185	156-220	140	127-154
Malachite green.....	0.26	0.22-0.31	0.11	0.09-0.14
Merthiolate.....	110	87-139	64.5	57.6-72.2
Methylene blue.....	51.0	40.2-64.8	33.0	26.2-41.6
Neguvon.....	78.0	64.5-94.4	71.0	55.9-90.2
Nickel sulfate.....	--	--	495	450-544
P.M.A.....	20.0	18.0-22.2	16.0	13.4-19.0
Quinacrine HCL.....	120	73-198	79.0	54.1-115.3
Roccal.....	2.10	1.94-2.27	1.68	1.56-1.81
Ruelene.....	36.0	34.3-37.8	35.0	33.0-37.1
Tiguvon.....	15.7	13.2-18.7	8.90	7.67-10.32
Trolene.....	2.50	2.25-2.78	1.00	0.67-1.50
TV-1096.....	37.0	33.3-41.1	28.2	25.9-30.7

species, and LC_{50} values range from 0.11 to 0.40 p.p.m. at 48 hours. Clemens and Sneed (1958a) reported its LC_{50} to channel catfish as 0.14 p.p.m. in 24 and 48 hours at 25°C. Our results show the LC_{50} values to be 0.21 and 0.20 p.p.m. in 24 and 48 hours respectively at 17°C. This variation between results may be due to differences in test temperatures.

Following malachite green in decreasing order of toxicity are Trolene, CoRa1, and Tiguvon, all of which have the basic structure of phosphorothioate. In the same general range of toxicity are Roccal and P.M.A., with Roccal the more toxic of the two. Roccal, like malachite green, exhibits relatively uniform toxicity, and LC_{50} values range from 1.12 to 3.40 p.p.m. at 48 hours for all species.

P.M.A. exhibits a much wider range of toxicity with LC_{50} values of 2.9 to 16.0 p.p.m. at 48 hours. Clemens and Sneed (1958a) reported its LC_{50} to channel catfish as 3.8 p.p.m. in 24 hours at 24°C. In a later publication, these authors (1958b) reported the LC_{50} of P.M.A. to channel catfish as 2.96 and 2.81 p.p.m. in 24 and 48 hours, respectively, at 16.5°C. Both reports compare favorably with our LC_{50} values of 3.22 and 2.89 p.p.m. for 24 and 48 hours at 17°C.

Acridlavine, amopyroquin dihydrochloride, merthiolate, methylene blue, Neguvon, Ruelene, and TV-1096 fall into an intermediate toxicity range with LC_{50} s of 10 to 100 p.p.m. Only Ruelene exhibits a uniform LC_{50} range of 25.7 to 35.0 p.p.m. for six species in 48 hours. The other compounds of this group demonstrate a relatively wide range of toxicity to the different species.

TV-1096 is soluble only to approximately 30 p.p.m. in water. Amounts above this level produce a saturated solution with a precipitate on the bottom of the test vessel. Brown trout fail to succumb to concentrations below 30 p.p.m. and for this reason, LC_{50} values could not be derived for the species. Also, amounts of TV-1096 in excess of a saturated solution are nontoxic to brown trout. In contrast, LC_{50} values of TV-1096 for the other species range from 16.1 to 28.2 p.p.m. at 48 hours.

Nickel sulfate, formalin, and quinacrine hydrochloride are the least toxic of the compounds analyzed. Formalin exhibits a fairly uniform LC_{50} range of 96 to 185 p.p.m. at 48 hours. The other two have a much wider range.

Clemens and Sneed (1958a) reported the LC_{50} values of formalin on channel catfish to be 87 and 69 p.p.m. in 24 and 48 hours, respectively, at 25°C whereas we found them to be 137 and 96 p.p.m. in 24 and 48 hours, respectively, at 17°C. This variation in results seems to indicate that the toxicity of formalin may be increased by an increase in temperature. The observation is supported by our results which show that formalin is more toxic to channel catfish at 17°C than it is to four species of trout and to bluegills at 12°C.

Erythromycin thiocyanate and quinine hydrochloride were tested at an arbitrary level of 100 p.p.m. Their solubility would have permitted higher concentrations but preliminary tests indicated little toxicity. At 100 p.p.m., the substances were not toxic to the fish.

The poor solubility of Flagyl, sulfamerazine, sulfamethazine, and sulfisoxazole prevented the determination of LC_{50} values. Solutions were saturated before lethal levels could be reached. The arbitrary concentration of 100 p.p.m. was selected then for tests. This resulted in saturated solutions with excess chemical remaining on the bottom of the bioassay vessels. None of them was toxic to the six species of fish.

DISCUSSION

Malachite green has been in use for many years as a fungicidal dip for fish (Foster and Woodbury, 1936). Recently, Amlacher (1961) recommended it for prolonged treatment of fish in ponds to combat *Ichthyophthirius*, *Chilodenella*, and *Costia*. He applied 0.15 p.p.m. and allowed it to dissipate in the water. Concentrations of 0.11 to 0.40 p.p.m. in our bioassays proved toxic within 48 hours to the six species of fish tested. Thus, there is a risk with concentrations over 0.11 p.p.m. in long-term treatments.

Trolene, CoRal, and Tiguvon are under consideration as prolonged treatments for control of Ichthyophthirius. Tiguvon is the least toxic of these organophosphates to fish. This indicates that it may prove the most valuable of the group if minimum concentrations required for control of "Ich" are approximately the same for all three.

Roccal has been in use as a bactericide for many years (Fish, 1947). Putz (1964) reported its possible value in prolonged, indefinite treatments at 0.25 to 0.50 p.p.m. for Ichthyophthirius. In treatments such as this, the chemical attacks the free-living stages of "Ich". He did not say which formulation of the chemical he used, but 10-percent active is the formulation commonly used in hatcheries (Davis, 1956). We used 50-percent active, and upon converting from 10-percent active to 50-percent active, the treatment levels could be reduced to 0.05 and 0.10 p.p.m. This permits a comparison between treatment levels and toxicity which shows a 10-fold difference in concentrations.

P.M.A. has been of considerable value in combating bacterial and protozoan diseases (Davis, 1956). Evidence of its greater toxicity to rainbow trout than other trouts has been reported over the years (Foster and Olson, 1951; Rodgers et al., 1951; Wolf, 1951; Hammer, 1960). Allison (1957) reported variations in the toxicity of P.M.A. from lot to lot of chemical. We used only one lot of P.M.A. in this study, and the results support the earlier findings that it has greater toxicity to rainbow trout. For example, it was up to three times as toxic to rainbow trout as to brook trout. Channel catfish appear to be sensitive to the compound at 17°C.

Snieszko and Friddle (1948) used merthiolate (sulfo) as a disinfectant for rainbow trout eggs. Van Duijn (1956) cautioned against use of merthiolate as a fish bath since the compound is a mercurial and is certain to be toxic to fish in contact with it for some period. We find an extreme variation in its toxicity to different species. This is especially true at 24 hours where LC_{50} values range from 7.5 p.p.m. for channel catfish to 110.0 p.p.m. for brown trout. This variation diminishes some-

what at 48 hours, and lake trout become the most sensitive to the chemical. The LC_{50} at 48 hours for lake trout is 2.1 p.p.m. in contrast with 74.5 p.p.m. for brown trout. Variations in resistance such as this may make merthiolate extremely difficult to work with in routine treatments of several species.

Acriflavine, amopyroquin dihydrochloride, methylene blue, Neguvon, Ruelene, and TV-1096 are under consideration as prolonged, indefinite treatments for control of Ichthyophthirius. Putz (1964) reported that 3 p.p.m. of acriflavine shows promise against the parasite. Our results indicate that bluegills are the most sensitive to the compound with LC_{50} values of 18.0 and 13.5 p.p.m. at 24 and 48 hours respectively. Channel catfish are the most resistant with LC_{50} values of 43.5 and 33.2 p.p.m. at 24 and 48 hours.

Clemens and Sneed (1958a) reported the LC_{50} values of acriflavine on channel catfish at 24 and 48 hours to be 11.5 and 6.8 p.p.m., respectively, at 20°C. Our finding, is that it is only about one-fourth as toxic as that. Possible causes for the discrepancy are many. Among them are differences in water quality and temperature, differences in the condition of fish, and purity of the compound used. In addition to this unexplained variation in the toxicity of acriflavine, another factor warrants serious consideration in its use. Van Duijn (1956) reported sterility in both egg-laying and live-bearing aquarium fish. This is a temporary situation and normal fertility is restored after several months.

Amopyroquin dihydrochloride also shows promise as a prolonged, indefinite treatment at 0.05 to 0.10 p.p.m. for control of Ichthyophthirius (Putz, 1964). Our results show that its toxicity to all trout tested, with the exception of lake trout, is between 35 and 40 p.p.m. for 48 hours. Also, bluegills at 12°C, and channel catfish at 17°C are approximately as sensitive as lake trout. A treatment level of 0.1 p.p.m. would include a safety margin in use of more than a hundredfold even against these three species.

Van Duijn (1956) recommended methylene blue as a satisfactory control for Ichthyophthirius in aquariums. He used 2 to 4 p.p.m.

of it in a permanent bath at temperatures between 21 and 27°C. We found that rainbow trout are the most sensitive to the dye, and the LC_{50} is 16 p.p.m. at 48 hours. The most resistant species is channel catfish with an LC_{50} of 104 p.p.m. at 48 hours. The remaining species are intermediate in sensitivity with a 48-hour LC_{50} range of 22.9 to 34.0 p.p.m. Comparison of the use levels with toxicity levels indicates a good safety margin.

Neguvon and Ruelene are of approximately the same toxicity except in one very important respect. Neguvon has a marked difference between the 24-hour and 48-hour LC_{50} . The most striking example of this involves lake trout with LC_{50} values of 41 p.p.m. at 24 hours and 9 p.p.m. at 48 hours. Differences between the 24- and 48-hour LC_{50} values by a factor of at least two are common except with bluegills. For some unknown reason the difference with bluegills is only 78 to 71 p.p.m.

Ruelene provides a contrast with Neguvon because it exhibits approximately the same toxicity at 24 and 48 hours. In the case of lake trout, the 24- and 48-hour LC_{50} values are identical at 27 p.p.m. It is possible that Ruelene degrades very rapidly in the test vessel to a nontoxic level.

TV-1096 has toxicity comparable to that of Neguvon and Ruelene. Like Neguvon, it does not appear to degrade as rapidly as Ruelene.

Nickel sulfate is under consideration as a prolonged, indefinite treatment for control of Ichthyophthirius. Our results show that it is relatively low in toxicity when compared with the other compounds tested, but it has a fairly wide range of toxicity among the species tested. The LC_{50} values at 48 hours range from 75 p.p.m. for lake trout to 495 p.p.m. for bluegills. Twenty-four-hour tests of 50 to 275 p.p.m. on brook trout and 200 to 500 p.p.m. on bluegills did not cause death.

Allison (1957) reported use of formalin as a parasiticide in long-term treatments in ponds. He suggested 5 p.p.m. for Gyrodactylus and 15 p.p.m. for Trichodina and Ichthyophthirius. Our results show that formalin is relatively and uniformly low in toxicity when compared

with the other compounds tested. It does appear to increase in toxicity as temperatures rise from 17°C to 25°C. Even with this increase in toxicity, the compound retains a safety margin of at least sixfold at recommended use levels.

Van Duijn (1956) recommended use of quinacrine hydrochloride in treatment of stubborn cases of "Ich" in aquarium fish. The treatment consists of three applications of 1 p.p.m. at 48-hour intervals. This totals 3 p.p.m. if no degradation of the compound occurs. He also stated that this treatment should not be extended over long periods and that 8 to 10 days should be sufficient.

Our results show that lake trout are approximately 10 times as sensitive to quinacrine hydrochloride as the other trout and 3 or 4 times as sensitive as bluegills and channel catfish. The sensitivity is complicated by the fact that the toxicity to lake trout is quite erratic and some deaths occur over a wide range of concentrations. The 48-hour LC_0 of quinacrine hydrochloride for lake trout is approximately 10 p.p.m., the LC_{50} is 21 p.p.m., and the LC_{100} is 110 p.p.m. Some fish succumb to the chemical quickly and at comparatively low concentrations whereas the rest survive for long periods. Further evidence of this lingering is shown by the slight difference between the 24-hour LC_{50} of 28 p.p.m. and the 48-hour LC_{50} of 21 p.p.m. In contrast, there is a considerable difference between the 24- and 48-hour LC_{50} values obtained for the other species. A possible explanation is that there is considerable variation in resistance among lake trout individuals.

Van Duijn (1956) recommended use of quinine hydrochloride for treatment of Ichthyophthirius in aquarium fish. The treatment consists in adding 1 p.p.m. on 3 successive days, a final treatment level of 3 p.p.m. He cautioned against use of the treatment for long periods because of possible fertility problems. Our results show that 100 p.p.m. of the chemical in water are not toxic within 48 hours to the species tested.

Erythromycin thiocyanate has been used as a food additive for control of kidney disease in rainbow trout at 4.5 grams per 100 pounds of

fish per day for 21 days (Piper, 1961). Warren (1963) reported that it is toxic to rainbow trout at 500 mg. per kg. Our results show that 100 p.p.m. of the antibiotic in water is not toxic to the species tested within 48 hours.

Flagyl has been used in medicine as an antiprotozoal agent (Cutting, 1962). Putz (1964) reported experimental use of it at 1.5 p.p.m. for control of *Ichthyophthirius*. Our results show that Flagyl is nontoxic at 100 p.p.m. The finding is qualified somewhat since the compound is not immediately soluble at 100 p.p.m. It dissolves slowly, however, and is completely in solution within 48 hours.

Snieszko and Bullock (1957) reported use of sulfamerazine, sulfamethazine, and sulfisoxazole as food additives in the treatment of furunculosis at 8 to 10 grams per 100 pounds of fish per day for 10 to 20 days. Van Duijn (1956) recommended use of the sodium salt of sulfamerazine at 1 part per thousand as an effective cure for worm cataract in aquarium fish. In our water, sulfamerazine, sulfamethazine, and sulfisoxazole are not soluble at 100 p.p.m., and saturated solutions are not toxic to the six species within 48 hours.

All of our results were obtained with fish which were, to the best of our knowledge, healthy. They showed no signs of disease or physical injuries. The toxicity of these compounds to fish which are sick or in poor condition might be significantly different.

None of the compounds reported herein are cleared by the Food and Drug Administration and the Department of Agriculture for use on fish destined for human consumption. The data and discussion presented in this paper should not be construed as recommendations for use.

CONCLUSIONS AND SUMMARY

The toxicities of 22 therapeutic compounds to rainbow trout, brown trout, brook trout, lake trout, and bluegills at 12°C. and channel catfish at 17°C. were determined in 24- and 48-hour static bioassays.

LC₅₀ values for malachite green, the most toxic compound tested, range from 0.1 to 0.4 p.p.m. for all species tested. CoRal, P.M.A., Roccal, Tiguvon, and Trolene are less toxic than malachite green, but still rank relatively high in toxicity. Their LC₅₀ values range from approximately 0.5 to 10 p.p.m. for all species.

Acriflavine, amopyroquin dihydrochloride, merthiolate, methylene blue, Neguvon, Rue-lene, and TV-1096 are intermediate in toxicity. The LC₅₀ values range from approximately 10 to 100 p.p.m. for all species. Merthiolate has wide variations in toxicity to various species.

Formalin, nickel sulfate, and quinacrine hydrochloride have relatively low toxicities. The LC₅₀ values are usually above 100 p.p.m. Quinacrine hydrochloride is substantially more toxic to lake trout than to the other species.

No tests of erythromycin thiocyanate and quinine hydrochloride were made at concentrations above 100 p.p.m. They are not toxic within 48 hours at this concentration. Saturated solutions of Flagyl, sulfamerazine, sulfamethazine, and sulfisoxazole are also not toxic within 48 hours.

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19. Toxicity of Bayer 73 to Fish

By Leif L. Marking and James W. Hogan, Chemists

Bureau of Sport Fisheries and Wildlife
Fish Control Laboratories
La Crosse, Wis., and Warm Springs, Ga.



United States Department of the Interior, Stewart L. Udall, *Secretary*
Stanley A. Cain, *Assistant Secretary for Fish and Wildlife and Parks*
Fish and Wildlife Service, Clarence F. Pautzke, *Commissioner*
Bureau of Sport Fisheries and Wildlife, John S. Gottschalk, *Director*
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TOXICITY OF BAYER 73 TO FISH

Bh Leif L. Marking and James W. Hogan, Chemists
Fish Control Laboratories
La Crosse, Wis., and Warm Springs, Ga.

ABSTRACT.--Bayer 73, a molluscicide sold commercially as Bayluscide, is highly toxic to 18 species of freshwater fish. Various temperatures and water qualities in static bioassays do not influence the toxicity greatly, but pH variations in chemically buffered solutions do. The biodegradability, efficacy, and relative safety of Bayer 73 in aquatic situations indicate possible usefulness as a general fish toxicant.

Nitrosalicylanilides display intense biological activity against a broad spectrum of organisms. Taborsky et al. (1959) and Taborsky and Starkey (1962 and 1963) reported antimicrobial activity of salicylanilides. They found various levels of activity of nitro- and halo-substituted salicylanilides against fungi and bacteria, and concluded that certain positions on the salicylanilide molecule are more active than others.

Taborsky and Starkey (1962) also reported antitumoral activity of substituted salicylanilides. Molnar and Baron (1964) and Vinson et al. (1961) stated that salicylanilides possess germicidal properties and are efficacious as hospital sanitizers.

The molluscicidal activity of Bayer 73, 2', 5-dichloro-4'-nitrosalicylanilide, was first recognized by Gönnert and Schraufstätter (1958). Since then it has been tested thoroughly in the laboratory and field against snails which are intermediate hosts for schistosomiasis (Gönnert, 1961; Schraufstätter et al., 1961; Schraufstätter, 1962; Strufe and Gönnert, 1962; Foster, 1962; and Webbe, 1963). In addition, Gillet and Braux (1962) determined in laboratory tests with snails that Bayer 73 is ovicidal and cercaricidal, which enhances its effectiveness in breaking the cycle of schistosomes. Meyling et al. (1962) found that it killed 100 percent of the snail eggs at 1.0 p.p.m. in 4 to 5 hours.

Bayer 73 is relatively nontoxic to mammals. Hecht and Gloxhuber (1962) reported that dogs tolerated 0.1 g./kg. orally. Duhm (1963) indicated that humans and animals are not harmed by drinking water containing molluscicidal concentrations of Bayer 73. Foster (1962) and Holz and Hwa (1963) reported survival of plants exposed to molluscicidal concentrations. Shiff and Garnett (1961) reported reduced planktonic life immediately after application of 1.0 p.p.m. of Bayer 73, but the effect was short lived, and 32 days later populations were back to normal.

Gönnert (1962) and Webbe (1963) reported fish kills with Bayer 73 at a concentration of 0.3 p.p.m. Howell et al. (1964) found that Bayer 73 is highly toxic to sea lamprey larvae and rainbow trout. They further discovered that Bayer 73 synergized 3-trifluoromethyl-4-nitrophenol, and potentiated the compound as a selective lamprey larvicide.

In research for fish control chemicals, Walker et al. (1966) described the structure-activity relation of substituted salicylanilides to fish. Starkey and Howell (1966) showed that a number of substituted salicylanilides are more toxic to larval sea lampreys than rainbow trout. Marking et al. (in press) defined the toxicity of 3'-chloro-3-nitrosalicylanilide to larval sea lamprey and many species of

fish in the laboratory under standard test conditions and also in simulated stream environments. The compound is highly toxic to all species but most toxic to larval lamprey and several species of rough fish.

In view of the possible wide use of Bayer 73 in aquatic environments for controlling schistosomiasis and the piscicidal activity previously reported, there is a need to define its toxicity to fish and evaluate its potential in controlling fish populations. Accordingly, Bayer 73 was bioassayed at the Fish Control Laboratories at La Crosse, Wis., and Warm Springs, Ga., to determine its effects on fish in various laboratory environments.

MATERIALS AND METHODS

The sample (control No. C910) was obtained from Chemagro Corporation, Kansas City, Mo. as a technical formulation containing 99 percent 2-aminoethanol salt of 2', 5-dichloro-4-nitrosalicylanilide. Its structure is illustrated in figure 1. The material is a bright yellow, crystalline powder.

Test fish were obtained from National, State, and private fish hatcheries (table 1) and were acclimated to test conditions ac-

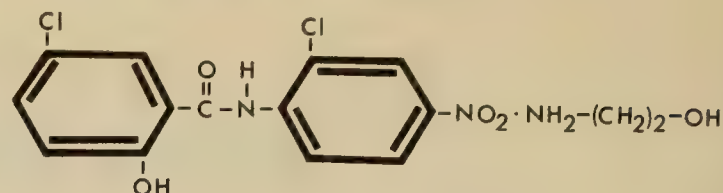


Figure 1.--Structure of 2-aminoethanol salt of 2', 5-dichloro-4'-nitrosalicylanilide.

cording to procedures described by Lennon and Walker (1964).

Preliminary screening was in 15 liters of bioassay medium at 12° C. at La Crosse and at 17° C. at Warm Springs. The fish were exposed to 0.1, 1.0, and 10.0 p.p.m. Bayer 73 to determine the general range of toxicity and the rate of reaction at the higher concentrations.

Delineative tests, including variations in test media such as temperature, water quality, and pH were conducted using 10 fish per concentration and at least 9 concentrations per test, plus a control. Temperature variations were maintained mechanically, and water quality variations were obtained by adding selected quantities of reconstitution salts to de-ionized water (table 2).

TABLE 1.--Sizes and sources of test fish

Species	Lot	Average length (inches)	Average weight (grams)	Source
Rainbow trout, <i>Salmo gairdneri</i>	279	1.9	1.0	Nevin SFH, Wis.
Brook trout, <i>Salvelinus fontinalis</i>	295	1.8	1.0	Osceola SFH, Wis.
Goldfish, <i>Carassius auratus</i>	W116	1.7	1.3	Tallassee PFH, Ala.
Do.....	W96A	1.6	1.3	Marion NFH, Ala.
Carp, <i>Cyprinus carpio</i>	W78	1.7	1.1	Marion NFH, Ala.
Do.....	W86	2.0	1.9	Marion NFH, Ala.
Do.....	W89	2.3	2.2	Marion NFH, Ala.
Do.....	367	1.5	0.8	Genoa NFH, Wis.
Fathead minnow, <i>Pimephales promelas</i>	W113	1.8	1.0	Marion NFH, Ala.
White sucker, <i>Catostomus commersoni</i>	381	2.0	1.2	Lake Mills NFH, Wis.
Bigmouth buffalo, <i>Ictiobus cyprinellus</i>	W79	2.0	1.5	Marion NFH, Ala.
Black bullhead, <i>Ictalurus melas</i>	393	1.9	1.3	Guttenberg NFH, Iowa
Brown bullhead, <i>Ictalurus nebulosus</i>	W148	1.8	1.1	Marion NFH, Ala.
Channel catfish, <i>Ictalurus punctatus</i>	W74A	3.0	2.8	Marion NFH, Ala.
Do.....	W87	2.8	3.0	Marion NFH, Ala.
Flathead catfish, <i>Pylodictis olivaris</i>	W154	2.2	1.1	Marion NFH, Ala.
Green sunfish, <i>Lepomis cyanellus</i>	W75	1.6	1.4	Marion NFH, Ala.
Bluegill, <i>Lepomis macrochirus</i>	W119	1.6	1.1	Marion NFH, Ala.
Do.....	340	1.5	0.6	Lake Mills, NFH, Wis.
Redear sunfish, <i>Lepomis microlophus</i>	W91	1.6	0.9	Marion NFH, Ala.
Smallmouth bass, <i>Micropterus dolomieu</i>	W146	1.6	0.7	Mammoth Springs NFH, Ark.
Largemouth bass, <i>Micropterus salmoides</i>	W140	1.6	0.8	Welaka NFH, Fla.
Yellow perch, <i>Perca flavescens</i>	382	1.7	0.6	Lake Mills NFH, Wis.
Tilapia, <i>Tilapia mossambica</i>	W156	1.5	0.8	Marion NFH, Ala.

TABLE 2.--Quality and composition of reconstituted water used at the Fish Control Laboratories

Classification of water	Salt added in mg. per liter				pH range	Concentration in p.p.m. CaCO ₃ as total	
	NaHCO ₃	CaSO ₄	MgSO ₄	KCl		hardness	alkalinity
Soft.....	12	7.5	7.5	0.50	6.4-6.8	10- 13	10- 13
Medium ¹	48	30.0	30.0	¹ 2.00	7.2-7.6	40- 48	30- 35
Hard.....	192	120.0	120.0	8.00	7.6-8.0	160-180	110-120

¹ Standard reconstituted water used in routine bioassays.

Different buffers to control the pH of test media were prepared from reagent grade chemicals. The normal and molar solutions and corresponding dilutions are listed in table 3. The pH's of solutions were checked daily and adjusted as necessary to yield approximately the desired pH level.

The delineative data were analyzed according to the methods of Litchfield and Wilcoxon (1949) to determine LC₅₀ values, variation, slope functions, and 95-percent confidence intervals.

TABLE 3.--Volumes of buffer reagents (ml.) necessary to yield desired pH levels in 15 liters of test solution

Reagent and strength	Volume to yield pH of approximately--		
	5	7	10
Sodium hydroxide (NaOH).....1.0 N	10	10	20
Potassium acid phthalate (KHC ₈ H ₄ O ₄).....0.1 M	510	--	--
Potassium phosphate (KH ₂ PO ₄).....1.0 M	--	30	--
Boric acid (H ₃ BO ₃).....1.0 M	--	--	21

RESULTS

PRELIMINARY SCREENING

Initial tests with Bayer 73 indicated a high level of biological activity in fish. In a concentration of 0.1 p.p.m., all rainbow trout succumbed in 15 minutes, flathead catfish died within an hour, and yellow perch died within 24 hours, but not all white suckers, black bullheads and bluegills died within the 96-hour bioassay. Exposure to a 1.0 p.p.m. concentration for 15 minutes caused deaths of all white suckers, flathead catfish, green sunfish,

and yellow perch. Carp, black bullheads, and bluegills were more resistant, but died during a 15-minute exposure to a concentration of 10.0 p.p.m.

The response of fish to the chemical is noticeable immediately after exposure to the higher concentrations. They appear irritated, and become excited when exposed to sound or external movements. Their early reactions include rapid, irregular respiration. Swimming is aimless, and some fish skitter on the surface while losing equilibrium. Just before death, respiration and swimming actions gradually slow and become irregular, and finally are spastic. Often the opercula are extended on the dead fish.

DELINEATIVE SCREENING

General toxicity.--Bayer 73 is toxic to the 18 species of fish tested. The LC₅₀ values, 95-percent confidence intervals, and mean slope functions for routine tests are given in table 4 for the 24- to 96-hour exposure periods. Exposure beyond 24 hours produces little additional mortality. In fact, the figures indicate no difference in the 24- and 48-hour LC₅₀ values because the confidence intervals overlap for all species at the two exposure periods. The LC₅₀ values for brook trout, one lot of carp, and brown bullheads remained the same from 24- to 96-hour exposures.

The 95-percent confidence intervals of 24- to 96-hour LC₅₀'s overlap for all species except brown bullhead, redear sunfish, small-mouth bass, largemouth bass and tilapia. Intervals are narrow in most cases, indicating consistent toxic effects with little range between concentrations resulting in survival and death.

TABLE 4.--Toxicity of Bayer 73 to selected species of fish

Species	Temp. C.	LC ₅₀ (p.p.m.) and 95-percent confidence interval			Mean slope function
		24 hours	48 hours	96 hours	
Rainbow trout.....	12°	0.052 0.049-0.056	0.052 0.049-0.056	0.050 0.047-0.054	1.133
Brook trout.....	12°	0.061 0.058-0.065	0.061 0.058-0.065	0.061 0.058-0.065	1.070
Goldfish.....	17°	0.279 0.243-0.321	0.279 0.243-0.321	0.230 0.201-0.263	1.147
Carp.....	12°	0.143 0.133-0.154	0.139 0.134-0.145	0.139 0.134-0.145	1.053
Do.....	17°	0.148 0.142-0.159	0.148 0.142-0.159	0.148 0.142-0.159	1.060
Do.....	17°	0.245 0.227-0.265	0.235 0.214-0.258	0.225 0.208-0.243	1.106
Fathead minnow.....	17°	0.106 0.097-0.116	0.103 0.096-0.111	0.102 0.089-0.117	1.106
White sucker.....	12°	0.084 0.073-0.097	0.081 0.075-0.088	0.081 0.076-0.086	1.084
Bigmouth buffalo.....	17°	0.080 0.059-0.108	0.064 0.056-0.073	-	1.245
Black bullhead.....	12°	0.104 0.084-0.123	0.096 0.087-0.106	0.088 0.078-0.098	1.156
Brown bullhead.....	17°	0.071 0.065-0.077	0.071 0.066-0.077	0.056 0.049-0.064	1.107
Channel catfish.....	17°	0.084 0.079-0.089	0.084 0.079-0.089	0.082 0.077-0.088	1.063
Flathead catfish.....	17°	0.043 0.040-0.046	0.043 0.040-0.046	0.043 0.040-0.046	1.060
Green sunfish.....	17°	0.158 0.145-0.172	0.115 0.101-0.130	0.100 0.094-0.107	1.097
Bluegill.....	12°	0.105 0.095-0.116	0.098 0.085-0.112	0.094 0.083-0.107	1.131
Do.....	17°	0.082 0.072-0.092	0.082 0.070-0.096	0.068 0.057-0.080	1.158
Redear sunfish.....	17°	0.157 0.148-0.167	0.153 0.142-0.165	0.088 0.086-0.090	1.069
Smallmouth bass.....	17°	0.089 0.085-0.099	0.089 0.085-0.099	0.060 0.048-0.074	1.223
Largemouth bass.....	17°	0.111 0.099-0.124	0.097 0.087-0.109	0.062 0.050-0.076	1.157
Yellow perch.....	12°	0.082 0.076-0.088	0.081 0.069-0.087	0.081 0.069-0.087	1.077
Tilapia.....	17°	0.180 0.159-0.203	0.150 0.133-0.170	0.109 0.090-0.132	1.157

The slope functions were consistent in exposures of 24, 48, and 96 hours, and they were averaged to yield the figures given in table 4. The values, ranging from 1.060 to 1.245, are low, the regression is nearly vertical, and there is little difference between concentration permitting complete survival and that producing complete mortality. In contrast, the slope function of p,p'-DDT for goldfish is 6.02 (Marking, 1966).

Comparative toxicity.--Bayer 73 is highly toxic to all species, with 96-hour LC_{50} values ranging from 0.043 to 0.230 p.p.m. Flathead catfish, rainbow trout, brown bullheads, smallmouth bass, brook trout, and largemouth bass in that order are the most sensitive species to Bayer 73 (fig. 2). Goldfish are the most resistant, followed by carp. Intermediate in sensitivity are white suckers, yellow perch, channel catfish, black bullheads, redear sunfish, bluegills, green sunfish, tilapia, and fathead minnows. Bayer 73 does not appear spe-

cific to any undesirable species of fish but rather is generally toxic to game and rough fish.

Short exposures.--Among the six species selected to determine effects of short-term exposures to Bayer 73, rainbow trout were most sensitive and carp most resistant (table 5). Rainbow trout, brook trout, and yellow perch respond rather quickly; LC_{50} values are only slightly greater at 1 hour than at 6 hours. The 95-percent confidence intervals overlap for each species in the 2-, 3-, and 6-hour exposures. Also, the values at 6 hours and 24 hours for rainbow trout and brook trout do not differ significantly (tables 5 and 6).

Carp, white suckers, and black bullheads respond somewhat more slowly at 1 hour, although their resistance is greater initially and the increments in LC_{50} values from 1- to 96-hour exposures are small and fairly uniform.

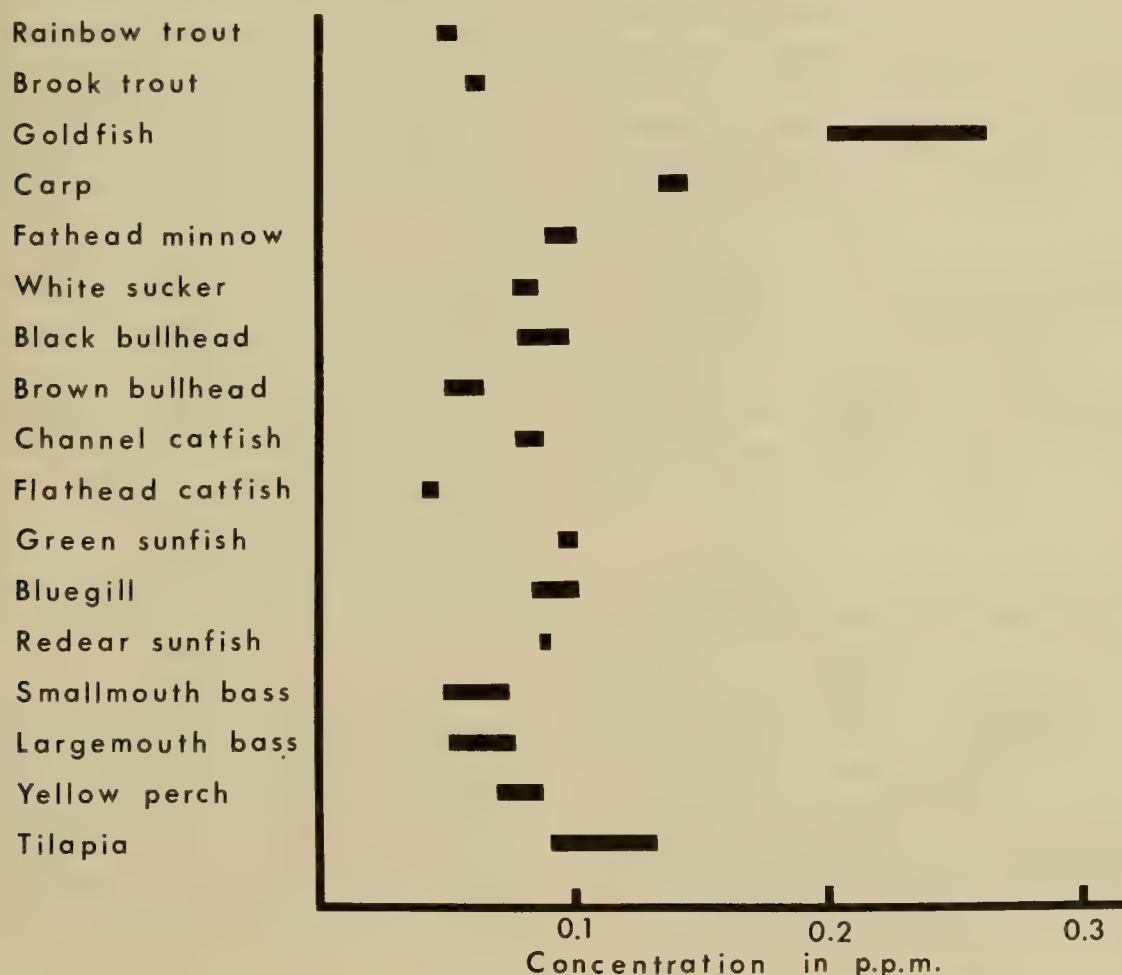


Figure 2.--95-percent confidence intervals for LC_{50} values of Bayer 73 to 17 species of fish. The data was taken from table 4 at 96-hour exposures.

TABLE 5.--Toxicity of Bayer 73 in short exposures at 12° C.

Species	LC ₅₀ (p.p.m.) and 95-percent confidence interval at--				Mean slope function
	1 hour	2 hours	3 hours	6 hours	
Rainbow trout.....	0.063 0.060-0.066	0.056 0.053-0.059	0.052 0.049-0.056	0.052 0.049-0.056	1.064
Brook trout.....	0.077 0.069-0.086	0.069 0.063-0.076	0.064 0.060-0.068	0.064 0.060-0.068	1.089
Carp.....	0.300 0.267-0.338	0.250 0.232-0.264	0.200 0.188-0.214	0.188 0.173-0.204	1.080
White sucker.....	0.180 0.177-0.183	0.108 0.092-0.127	0.105 0.091-0.122	0.100 0.088-0.114	1.126
Black bullhead....	0.275 0.230-0.329	0.210 0.181-0.243	0.156 0.138-0.177	0.156 0.138-0.177	1.156
Yellow perch.....	0.120 0.103-0.140	0.116 0.103-0.131	0.116 0.101-0.133	0.100 0.089-0.112	1.143

TABLE 6.-- Effects of temperature on toxicity of Bayer 73 to fish

Species	Temp. C.	LC ₅₀ (p.p.m.) and 95-percent confidence interval at--				
		3 hours	6 hours	24 hours	48 hours	96 hours
Rainbow trout.....	7°	0.059 0.053-0.066	0.050 0.044-0.057	0.048 0.042-0.055	0.048 0.042-0.055	0.047 0.043-0.052
Do.....	12°	0.052 0.049-0.056	0.052 0.049-0.056	0.052 0.049-0.056	0.052 0.049-0.056	0.050 0.047-0.054
Do.....	17°	0.054 0.048-0.059	0.053 0.048-0.058	0.052 0.048-0.057	0.052 0.048-0.057	0.050 0.048-0.054
Goldfish.....	12°	0.340 0.315-0.367	0.270 0.250-0.290	0.228 0.207-0.251	0.228 0.207-0.251	0.228 0.207-0.251
Do.....	17°	0.350 0.311-0.394	0.283 0.258-0.311	0.279 0.243-0.321	0.279 0.243-0.321	0.230 0.201-0.263
Do.....	22°	0.290 0.260-0.320	0.268 0.237-0.302	0.252 0.221-0.287	0.206 0.181-0.235	0.223 0.179-0.279
Channel catfish....	12°	0.065 0.060-0.070	0.056 0.053-0.059	0.052 0.048-0.056	0.050 0.046-0.054	0.049 0.045-0.053
Do.....	17°	-	-	0.084 0.079-0.089	0.084 0.079-0.089	0.082 0.077-0.088
Do.....	22°	0.058 0.055-0.061	0.058 0.055-0.061	0.055 0.052-0.058	0.055 0.052-0.058	0.042 0.035-0.051
Bluegill.....	12°	0.141 0.128-0.155	0.115 0.103-0.129	0.106 0.094-0.120	0.092 0.085-0.099	0.092 0.085-0.099
Do.....	17°	0.120 0.107-0.135	0.096 0.082-0.110	0.082 0.072-0.092	0.082 0.070-0.096	0.068 0.057-0.080
Do.....	22°	0.100 0.093-0.108	0.092 0.080-0.106	0.082 0.065-0.103	0.066 0.058-0.076	0.054 0.046-0.063

The values for all 6 species vary from 0.063 to 0.300 p.p.m. in 1 hour to 0.050 to 0.139 in 96 hours. The figures indicate that fish die rather quickly at concentrations only slightly greater than those required to kill at 96-hour exposures.

The average slope functions for 1-, 2-, 3-, and 6-hour exposures are low and similar to those for longer exposures (table 5). The figures indicate a steep regression where small increases in concentration produce considerable additional mortality.

Effects of temperature.--Bayer 73 was tested with rainbow trout, goldfish, channel catfish, and bluegills at 7°, 12°, and 17° C. in 3-, 6-, 24-, 48-, and 96-hour exposures (table 6). These changes in temperature had no significant effect on the toxicity of Bayer 73 to rainbow trout.

Goldfish, although more resistant than rainbow trout, reacted similarly at 12°, 17°, and 22° C. The difference is not significant at any one temperature, but the data indicate greater toxicity at 22° than at 12° or 17° C. The confidence intervals overlap for the 6-, 24-, 48-, and 96-hour exposures but not for the 3-hour exposure.

The channel catfish tested at 17° were different from the lot tested at 12° and 22° C. The results indicate greater resistance at 17°, but the difference may be attributable to the source and physical condition of the fish. Another lot of channel catfish tested in 3- and 6-hour bioassays indicated little or no difference at 17°. The responses at 12° and 22° obtained from the same lot of catfish show no significant differences.

Bluegills respond to increases in temperature more than the other species (table 6). Bayer 73 is more toxic at 22° than at 12° or 17°. The confidence intervals do not overlap at 12° and 22° at exposures of 3, 48, and 96 hours. The increase in toxicity at longer ex-

posures also is more noticeable with bluegills than with the other three species.

Effects of water quality.--Bayer 73 was tested in soft, medium, and hard water prepared according to table 2. At all exposure periods, the chemical is more toxic to rainbow trout in soft water and less toxic in hard water (table 7). The greater decrease in toxicity is between medium and hard water and suggests degradation of Bayer 73 at higher pH values and higher alkalinities.

Carp respond much the same as rainbow trout, although the confidence intervals overlap at the extreme water quality variations (table 7). The carp tested in water of medium hardness were from a different lot of fish. The data indicate greater sensitivity in water of medium hardness than in soft water, but the difference is not significant.

The toxicity of Bayer 73 to channel catfish decreases significantly in hard water but changes very little from soft to medium hard water. The toxic effects are uniform within each hardness, and do not increase significantly from the 3-hour to the 96-hour exposure (table 7).

Bluegills also are more susceptible to Bayer 73 in softer waters (table 7). The LC_{50} values in hard water are 2 or more times those in medium water. Confidence intervals for medium and hard water do not overlap at any exposure.

Effects of pH.--Variations in pH were accomplished by adding buffering agents to standard test water according to table 3. There were significant differences in the toxicity of Bayer 73 to goldfish at pH 5, 7, and 10 (table 8). The tests at pH 7 conform somewhat to tests in standard bioassays although these goldfish are from a different lot and appear more sensitive to Bayer 73 than the goldfish tested in standard water.

TABLE 7.--Effects of water quality on toxicity of Bayer 73 to fish

Species	Water quality	LC ₅₀ (p.p.m.) and 95-percent confidence interval at--				
		3 hours	6 hours	24 hours	48 hours	96 hours
Rainbow trout....	soft	0.046 0.043-0.049	0.046 0.043-0.049	0.046 0.043-0.049	0.046 0.043-0.049	0.046 0.043-0.049
Do.....	medium	0.054 0.050-0.059	0.053 0.049-0.055	0.053 0.049-0.055	0.053 0.049-0.055	0.053 0.049-0.055
Do.....	hard	0.082 0.075-0.090	0.082 0.075-0.090	0.082 0.075-0.090	0.082 0.075-0.090	0.070 0.066-0.074
Carp.....	soft	-	-	0.197 0.168-0.230	0.188 0.181-0.196	0.191 0.170-0.214
Do.....	medium	-	-	0.169 0.163-0.178	0.169 0.163-0.178	0.169 0.163-0.178
Do.....	hard	-	0.342 0.309-0.374	0.229 0.210-0.250	0.229 0.207-0.242	0.224 0.207-0.242
Channel catfish..	soft	0.082 0.075-0.090	-	0.070 0.066-0.074	0.070 0.066-0.074	-
Do.....	medium	0.064 0.058-0.071	-	0.062 0.056-0.068	0.062 0.056-0.068	0.060 0.054-0.067
Do.....	hard	0.079 0.091-0.104	-	0.093 0.085-0.101	0.093 0.085-0.101	0.093 0.085-0.101
Bluegill.....	soft	0.092 0.084-0.101	0.083 0.074-0.093	0.083 0.074-0.093	0.073 0.060-0.088	0.064 0.055-0.074
Do.....	medium	0.120 0.106-0.135	0.096 0.082-0.110	0.082 0.072-0.092	0.082 0.070-0.096	0.068 0.057-0.080
Do.....	hard	0.200 0.183-0.219	0.190 0.170-0.212	0.178 0.152-0.209	0.154 0.136-0.175	0.117 0.100-0.137

TABLE 8.--Effects of pH on the toxicity of Bayer 73 to goldfish at 12° C.

pH	LC ₅₀ (p.p.m.) and 95-percent confidence interval at--			
	3 hours	24 hours	48 hours	96 hours
5...	4.600 3.880-5.460	3.520 3.120-3.980	3.500 3.100-4.000	3.500 3.100-4.000
7...	-	0.180 0.163-0.198	0.180 0.163-0.198	-
10...	-	6.420 5.780-7.130	6.150 5.690-6.640	3.190 2.850-3.580

DISCUSSION

Bayer 73 is toxic to fish in very brief exposures and does not become proportionately more toxic with increased exposure time. In several cases the LC₅₀ differed only little between 1 and 96 hours. This suggests rapid degradation of the toxicant either by the water or the fish. Duhm et al. (1963) found that rats

fed 50 mg./kg. 5,2'-dichloro-4'-nitro-salicylic-anilide excrete most of the material as 5,2'-dichloro-4'-aminosalicylic-anilide in a conjugated form. The amino-structures excreted are very unstable and decompose when the excrement is left to stand for several hours. They concluded that the chemical is reduced and detoxified by metabolism in rats. Quite possibly, fish are able to detoxify Bayer 73 by a reduction process similar to that in rats. This may explain to some extent why the comparatively high initial activity of the compound is followed by minimum time-concentration effects.

Strufe and Gönner (1962) reported that water temperature influences the efficacy of Bayer 73 in destroying snails. They found that 0.3 p.p.m. in 24-hour exposures is sufficient to kill all the snails at 20°, but 0.5 p.p.m. is necessary to yield 100-percent mortality at 5° C. They also indicated that solutions of Bayer 73 exposed to diffused daylight at 20°

to 50° C. for 9 hours show no significant loss of active ingredient. Our data indicate greater biological activity at higher temperatures, and the results probably would be more significant at extreme temperatures.

Gönnert (1962) reported that environmental factors such as salt content and pH of the water do not decisively influence activity of Bayer 73 against snails during the required exposure time. Strufe and Gönnert (1962) stated that the biological action of Bayer 73 against snails is not influenced to any measurable degree by salty waters containing up to 500 p.p.m. of calcium and magnesium. The laboratory tests with fish indicate less activity of the compound in harder water but, as other scientists report on snails, the difference is not considerable.

The pH variations in different water qualities which ranged from 6.4 to 8.0, did not influence toxicity drastically; the buffered pH 5 and 10 solutions did. Data from the latter test indicate less toxicity at low and high pH values and little difference at pH 7. The probable cause of decreased toxicity at extreme pH's may be a solubility or ionization factor in which less active chemical is available to the fish. Recently, Meyling and Pitchford (1966) found that as the pH of Bayer 73 solutions increase from 6.0 to 8.0 the solubility increases from less than 1.0 to 5.0 p.p.m. They did not define the solubility at pH 10. Strufe and Gönnert (1962) concluded from irradiation tests that up to 15 percent of the molluscicide is inactivated in neutral and weakly alkaline conditions, and up to 30 percent in the acid range of pH 5 to 6. Our tests at the extreme pH values of 5 and 10 confirm their findings.

Fox et al. (1963) reported that at pH 9.7 to 9.9 about 4 times as much Bayer 73 is required to kill snails as at pH 7.0 to 9.2. They suggest that snails require continuous stimulation by chlorine in order to bring into play their detoxifying mechanisms.

Meyling et al. (1962) reported that concentrations of Bayer 73 in hard water exposed to sunlight were reduced from 1.0 to 0.57 p.p.m. in 16 hours whereas in darkness the concentrations did not change after 312 hours. Ap-

parently sunlight, hard water, high pH, and alkalinity contribute to the reduction of Bayer 73.

Bayer 73 is highly toxic to certain fish species resistant to other chemicals. Catfishes, for example, are readily susceptible to it but are resistant to such other toxicants as antimycin A (Walker et al., 1964) and rotenone (Henegar, 1966).

Gönnert (1961) summarizes the results of laboratory and field trials with Bayer 73 in a number of countries. Minimum lethal concentrations for snails and snail ova were found to be between 0.2 and 0.5 p.p.m. Applications of 1.0 p.p.m. of Bayer 73 destroyed snails in standing and flowing water. He also indicated that the toxicity of Bayer 73 to fish is very similar to that for snails.

Our tests in the laboratory show that Bayer 73 produces 50-percent mortality in 18 species of fish at concentrations of 0.043 to 0.230 p.p.m. in standard 96-hour bioassays. More than 1.0 p.p.m. was required to kill fish only when the bioassays were chemically buffered to pH 5 and 10. Thus, in the laboratory, molluscicidal concentrations of Bayer 73 are toxic to all species of fish tested. Applications of 1.0 p.p.m. are probably toxic to fish in natural environments provided that extreme pH values of 5 or 10 do not prevail.

CONCLUSIONS

Bayer 73, a molluscicide, is highly toxic to 18 species of fish in static bioassays. Concentrations lethal to snails in the laboratory are also toxic to fish.

Bayer 73 kills 50 percent of the rainbow trout, brook trout, brown bullheads, flathead catfish, smallmouth bass, and largemouth bass at 0.062 p.p.m. or less in 96-hour exposures. Fathead minnows, white suckers, black bullheads, channel catfish, green sunfish, bluegills, redear sunfish, yellow perch, and tilapia are intermediate in sensitivity, and LC₅₀ values range from 0.81 to 0.109 p.p.m. in 96 hours. Goldfish and carp are more resistant species, with LC₅₀ values of 0.230 and 0.148 p.p.m.

The toxicity of Bayer 73 to bluegills increased with temperature, but the toxicity to trout, goldfish, and catfish was not significantly affected. Various water qualities also do not influence the toxicity greatly. Extreme pH changes significantly reduce the toxicity of Bayer 73 to fish. This effect may be due to decreased ionization at extreme pH values or to a metabolic reduction of the compound through stimulation of the detoxifying system in fish.

Because of its biodegradability and safety in water, Bayer 73 deserves consideration as a general fish toxicant.

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20. Toxicity of Dimethyl Sulfoxide (DMSO) to Fish

By Wayne A. Willford, Chemist

Bureau of Sport Fisheries and Wildlife
Fish Control Laboratory, La Crosse, Wis.



United States Department of the Interior, Stewart L. Udall, *Secretary*
Stanley A. Cain, *Assistant Secretary for Fish and Wildlife and Parks*
Fish and Wildlife Service, Clarence F. Pautzke, *Commissioner*
Bureau of Sport Fisheries and Wildlife, John S. Gottschalk, *Director*
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TOXICITY OF DIMETHYL SULFOXIDE (DMSO) TO FISH

By Wayne A. Willford, Chemist
Fish Control Laboratory, La Crosse, Wis.

ABSTRACT.--Toxicities of dimethyl sulfoxide (DMSO) to rainbow trout, brook trout, lake trout, carp, black bullhead, channel catfish, green sunfish, bluegill, and yellow perch were determined in 24-, 48-, and 96-hour static bioassays at 12° C. Toxicity was of low order, around 30 p.p.t. Water quality had little effect, but increased temperature increased the toxicity to rainbow trout. A preliminary test indicated that DMSO has little effect on the toxicity of antimycin to bluegill.

Dimethyl sulfoxide (DMSO) is the simplest of the homologous series of organic sulfoxides; it is prepared by oxidation of dimethyl sulfide. Its formula is $(CH_3)_2SO$, and the pure form is a hygroscopic, colorless, odorless liquid, melting at 18.45° and boiling at 189° C., with a specific gravity of 1.100 (Stecher, 1960).

DMSO was first synthesized in 1867 but remained a laboratory curiosity until the 1940's when it was successfully used as a solvent for the spinning of polyacrylonitrile fibers (Block, 1964). The first physiochemical data on the compound appeared in 1948, and since that time DMSO has been used extensively in several fields.

DMSO has been shown to possess remarkable potential as a solvent for many types of inorganic and organic compounds including gases (Willson et al., 1965). It has also been used as a preservative during freeze storage of red blood cells (Lovelock and Bishop, 1959), platelets (Geisler et al., 1964), spermatozoa (Sherman, 1964), mitochondria (Greiff, 1961), protozoa (Hwang, 1964), bone marrow (Ashwood-Smith, 1961a; Persidsky and Richards, 1963), cardiac muscle (Levy et al., 1962), and tissue culture cells (Dougherty, 1962; Porterfield and Ashwood-Smith, 1962). DMSO also exhibited radio-

protective action against lethal doses of X-irradiation in mice (Ashwood-Smith, 1961b).

In the medical field, the compound is under investigation as a penetrant carrier, local analgesic, anti-inflammatory adjunct, bacteriostatic agent, diuretic, tranquilizer, and potentiator (Jacob et al., 1964a). It is even reported to be good for headaches (Jacob, 1965). The most promising of these areas appears to be the ability of DMSO to penetrate biological membranes and act as a carrier for other drugs (Horita and Weber, 1964; Jacob et al., 1964b; Stoughton and Fritsch, 1964; Stoughton, 1965).

In the agricultural field, DMSO has been shown to be of value as a solvent carrier for certain compounds used in the control of plant diseases (Bean, 1965; Keil et al., 1965). It also has some herbicidal activity when used by itself for the control of purple nutsedge (Anderson and Dunford, 1966).

Additional information and references on DMSO can be obtained from articles written by Rosenkrantz et al. (1963), and Kligman (1965a and 1965b).

These interesting properties of DMSO plus the fact that it is miscible in all proportions with water suggested that it might be useful

in fisheries as a nontoxic solvent in toxicity studies and for the administration of non-water-soluble drugs to fish. In addition, if DMSO enhances the absorption by fish of compounds dissolved in it, a major breakthrough in the fields of fish control and fish disease control would result. It is for these purposes that the following toxicity studies were undertaken.

MATERIALS AND METHODS

Samples of DMSO were obtained from Ayerst Laboratories, New York, N.Y. The formulation was 90 percent DMSO and 10 percent water.

The fish were obtained from several fish hatcheries (table 1), and were introduced to the static bioassays after routine acclimation as described by Lennon and Walker (1964).

Preliminary bioassays were conducted in 1-gallon glass jars each containing 3 liters of bioassay media and two fish. After the approximate level of toxicity had been established, delineative bioassays were conducted in 5-gallon glass jars each containing 15

liters of reconstituted deionized water and 10 fish. The proper volume of bioassay media was maintained by removal of a quantity of water equal to the aliquot of DMSO which was to be added. Each test included 5 to 9 concentrations of chemical and 50 to 90 test fish plus 10 fish for controls.

Various water qualities were obtained by adding selected concentrations of reconstituting salts to deionized water (table 2). Tests were maintained at 7°, 12°, or 17° C. by water baths.

Survival and mortality were recorded at 24, 48, and 96 hours. The data were analyzed by plotting concentration versus mortality on logarithmic normal (probability) graph paper to define the concentration which produced 50-percent mortality (LC_{50}), slope function, variation, and 95-percent confidence intervals (C.I.) as described by Litchfield and Wilcoxon (1949).

All results are reported in parts per thousand (p.p.t.), by volume, of total material added to the test vessel instead of active ingredient.

TABLE 1.--Species, sizes, and sources of bioassay fish

Species	Average length (inches)	Average weight (grams)	Source ¹
Rainbow trout, <i>Salmo gairdneri</i>	1.8	0.9	NFH, Manchester, Iowa.
Do.....	1.8	1.0	Rainbow Ranches, Spokane, Wash.
Brook trout, <i>Salvelinus fontinalis</i>	1.5	0.5	SFH, Osceola, Wis.
Lake trout, <i>Salvelinus namaycush</i>	1.9	0.7	SFH, St. Croix Falls, Wis.
Carp, <i>Cyprinus carpio</i>	1.6	1.0	NFH, Lake Mills, Wis.
Black bullhead, <i>Ictalurus melas</i>	2.0	1.7	NFH, New London, Minn.
Channel catfish, <i>Ictalurus punctatus</i>	2.0	1.1	NFH, Fairport, Iowa.
Green sunfish, <i>Lepomis cyanellus</i>	1.5	0.8	NFH, Lake Mills, Wis.
Bluegill, <i>Lepomis macrochirus</i>	1.3	0.5	Do.
Yellow perch, <i>Perca flavescens</i>	2.6	1.7	Do.

¹ NFH = National Fish Hatchery; SFH = State Fish Hatchery.

TABLE 2.--Composition and analysis of reconstituted, deionized water used in bioassays

Classification of water	Amount of salts added (mg./l.)				pH range	Range of total hardness as p.p.m. $CaCO_3$	Range of total alkalinity as p.p.m. $CaCO_3$
	$NaHCO_3$	$CaSO_4$	$MgSO_4$	KCL			
Soft.....	12.0	7.5	7.5	0.5	6.4 - 6.8	10-13	10-13
Medium ¹	48.0	30.0	30.0	2.0	7.2 - 7.6	40-48	30-35
Hard.....	192.0	120.0	120.0	8.0	7.6 - 8.0	160-180	110-120

¹ Standard reconstituted water used in routine bioassays.

RESULTS AND DISCUSSION

PRELIMINARY TESTING

Preliminary tests to determine the approximate level of DMSO toxicity were carried out with yellow perch as the test species. In order to preserve the supply of test material it was necessary to prepare a 3-liter solution containing 500 p.p.t. of DMSO. Subsequent concentrations were obtained by dilution of this solution.

Results in terms of the approximate time to death at each concentration were as follows:

- 500 p.p.t. - 10 minutes
- 250 p.p.t. - 30 minutes
- 125 p.p.t. - 1.5 hours
- 62 p.p.t. - 24 hours
- 31 p.p.t. - no mortality within 96 hours

From these data, the 24-, 48-, and 96-hour LC₅₀ values appear to lie between 30 and 60 p.p.t., and concentrations were selected accordingly for the routine toxicity bioassays.

GENERAL TOXICITY

DMSO exhibited a consistent and nonselective toxicity of very low order to the nine species tested (table 3).

Probit analysis yielded slope functions on logarithmic paper which ranged from 1.06 to 1.20 with a mean of 1.11 on all tests. This

indicates that as the level of acute toxicity is approached a minimal change in concentration is required to produce either 0- or 100-percent mortality.

The comparative resistance of the nine species was extremely close. The 96-hour LC₅₀'s ranged from only 32.3 to 43.0 p.p.t. for the various species.

The order of susceptibility of the nine species varied with the observation period. At 24 and 48 hours, for example, channel catfish were the most susceptible, with bluegill the most resistant at 24 hours and yellow perch the most resistant at 48 hours. By 96 hours, rainbow trout became the most susceptible and green sunfish the most resistant. This fluctuation in the comparative order of sensitivity further exemplifies the extremely nonselective toxicity to all fish tested.

Recent studies at the Western Fish Nutrition Laboratory with yearling coho salmon (*Oncorhynchus kisutch*) determined the median tolerance limit (TLM) to be 72, 55, and 46 p.p.t. at 24, 48, and 96 hours respectively.¹ Ball (1966) reported that the 48-hour LC₅₀ of DMSO to goldfish (*Carassius auratus*) is 43 p.p.t. at 15° C. These results are in close agreement with the results at this laboratory and serve to further substantiate the consistent and nonselective toxicity of DMSO.

¹Personal communication from Pete Benville, Jr., Chemist, Western Fish Nutrition Laboratory, Bureau of Sport Fisheries and Wildlife, Cook, Wash., 1966.

TABLE 3.--Toxicity of 90-percent DMSO to nine species of fish at 12° C.

Species	At 24 hours		At 48 hours		At 96 hours	
	LC ₅₀ (p.p.t.)	95-percent C.I.	LC ₅₀ (p.p.t.)	95-percent C.I.	LC ₅₀ (p.p.t.)	95-percent C.I.
Rainbow trout.....	53.0	48.6 - 57.8	41.7	39.3 - 44.2	32.3	30.2 - 34.6
Brook trout.....	54.5	50.9 - 58.3	46.0	42.2 - 50.1	36.5	33.2 - 40.2
Lake trout.....	47.8	42.3 - 54.0	38.2	35.4 - 41.3	37.3	35.2 - 39.5
Carp.....	44.0	39.3 - 49.3	44.0	39.3 - 49.3	41.7	36.3 - 48.0
Black bullhead.....	42.5	37.9 - 47.6	39.2	35.3 - 43.5	36.5	33.8 - 39.4
Channel catfish.....	39.0	36.1 - 42.1	34.5	31.7 - 37.6	32.5	29.8 - 35.4
Green sunfish.....	65.0	61.3 - 68.9	52.5	47.7 - 57.8	43.0	35.8 - 51.6
Bluegill.....	72.0	63.2 - 82.1	56.0	51.9 - 60.5	33.5	29.9 - 37.5
Yellow perch.....	65.0	61.3 - 68.9	57.0	52.3 - 62.1	37.0	33.9 - 40.3

Rabinowitz and Myerson (1966) stated that a concentration of 19 p.p.t. of DMSO produced an approximate 48-hour LD₅₀ with neon tetras (*Paracheirodon innesi*), platys (*Xiphophorus maculatus*), mollies (*Pescilia latipinna*), and guppies (*Poecilia reticulata*). The LD₅₀'s for zebras (*Brachydanio rerio*) and catfish (*Corydoras paleatus*) were somewhere in excess of 25 p.p.t. These results were obtained in distilled water at 24° to 25° C. and the increased toxicity indicated may be the result of osmotic stress induced by the test media.

EFFECT OF WATER QUALITY AND TEMPERATURE ON TOXICITY

Changes in water quality at 12° C. had little or no effect upon the toxicity of DMSO (table 4). The LC₅₀ confidence interval in any particular water quality overlaps the LC₅₀'s in other water qualities within the same observation period in all cases except one. This exception was in hard water at 96 hours. In general, it appears that DMSO is slightly less toxic in hard water than it is in waters of soft or medium hardness.

Changes in temperature at medium hardness exhibited a substantial influence on toxicity (table 4). An increase in toxicity in excess of 10 p.p.t. as the temperature increases from 7° to 17° C. was observed at all observation periods.

This increase in toxicity at warmer temperature is in agreement with observations made at the Western Fish Nutrition Laboratory.

EFFECT OF DMSO ON TOXICITY OF ANTIMYCIN

A preliminary test was performed to determine what, if any, influence DMSO has on the toxicity of antimycin to bluegill. Various concentrations of antimycin were added in combination with enough DMSO to produce 1.0 p.p.t. of DMSO in the test vessel. A comparison test was run using only acetone as solvent for the antimycin.

The 96-hour LC₅₀ of antimycin and acetone alone was 0.089 parts per billion (p.p.b.), while antimycin in combination with 1.0 p.p.t. of DMSO produced a 96-hour LC₅₀ of 0.084 p.p.b. These results reflect biological variation and indicate that DMSO has no effect on the toxicity of antimycin at 96 hours. It is possible that in a bioassay designed to yield toxicity with shorter exposures, DMSO could enhance the absorption of antimycin sufficiently to affect toxicity.

Ball (1966) compared the relative toxicity of 0.05 p.p.m. *p,p'*-DDT to goldfish when used in combination with 6 and 18 p.p.t. of either DMSO or acetone. His results indicated that DMSO does not significantly affect the median survival time of goldfish when compared to acetone. He further suggested that DMSO may be a better solvent than acetone for pesticide toxicity studies.

Rabinowitz and Myerson (1966) were unable to show a significant difference in the uptake by aquarium fish of radioactive labeled dyes when used in combination with 1.0 p.p.t. of DMSO.

TABLE 4.--Effect of water quality and temperature on toxicity of DMSO (90-percent) to rainbow trout

Temperature C.	Water quality	At 24 hours		At 48 hours		At 96 hours	
		LC ₅₀ (p.p.t.)	95-percent C.I.	LC ₅₀ (p.p.t.)	95-percent C.I.	LC ₅₀ (p.p.t.)	95-percent C.I.
7°.....	medium	65.5	57.0-75.3	46.0	41.8-50.6	41.5	37.7-45.6
12°.....	medium	53.0	48.6-57.8	41.7	39.3-44.2	32.3	30.2-34.6
17°.....	medium	41.5	37.7-45.6	35.0	31.8-38.5	27.7	25.0-30.7
12°.....	soft	53.5	49.1-58.3	42.3	38.4-46.5	33.5	30.7-36.5
12°.....	hard	57.0	51.8-62.7	44.8	41.5-48.4	38.0	35.8-40.3

All of these results indicate that DMSO, when used as a diluted constituent, does not affect the absorption of some chemicals by fish. It has been shown to be an excellent solvent, and is worthy of further investigation as such for certain chemicals used in fisheries. In addition, the potential of DMSO as a penetrant carrier of certain drugs used in human medicine suggests investigation of similar potential in the treatment of fish disease.

CONCLUSIONS

1. The acute toxicity of DMSO to fish is of a very low order.
2. When the level of acute toxicity is reached, DMSO is abruptly and nonselectively toxic to the nine species tested.
3. Various water qualities at 12° C. have little effect upon the toxicity of DMSO to rainbow trout.
4. Increases in temperature cause a definite increase in the toxicity of DMSO to rainbow trout.
5. Preliminary results indicate that 1.0 p.p.t. of DMSO has no effect on the toxicity of antimycin to bluegill at 96 hours.

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21. Labor-saving Devices for Bioassay Laboratories

By Robert J. Hesselberg, Chemist and
Ralph M. Burress, Fishery Biologist

Southeastern Fish Control Laboratory
Bureau of Sport Fisheries and Wildlife
Warm Springs, Georgia



United States Department of the Interior, Stewart L. Udall, *Secretary*
Stanley A. Cain, *Assistant Secretary for Fish and Wildlife and Parks*
Fish and Wildlife Service, Clarence F. Pautzke, *Commissioner*
Bureau of Sport Fisheries and Wildlife, John S. Gottschalk, *Director*
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LABOR-SAVING DEVICES FOR BIOASSAY LABORATORIES

By Robert J. Hesselberg, Chemist
and Ralph M. Burrell, Fishery Biologist
Southeastern Fish Control Laboratory
Warm Springs, Ga.

ABSTRACT.--Three inexpensive pieces of labor-saving apparatus for bioassay laboratory use are described and illustrated. Construction features, material costs, and use of a jar rinser, automatic liquid measuring vessel, and jar emptier are discussed.

Nearly all of the indoor bioassays at the Fish Control Laboratories require the use of 5-gallon glass jars. Considerable work is involved in handling the scores of jars used daily, and much thought and effort have been given to developing methods to reduce the labor.

In recent months three labor-saving devices were developed and put into use: a rack for simultaneously rinsing 26 jars, a vessel for automatically measuring 15 liters of water into each jar, and a simple apparatus for emptying jars. Hundreds of man-hours of work are saved, and the hazards in handling heavy, slippery jars are reduced. The devices are not expensive and may be adapted to meet needs which differ from our own.

JAR RINSING RACK

DESCRIPTION

The essential features of the rack are shown in figure 1. It is about 15 feet 6 inches long, 28 inches wide, and 18 inches high, but dimensions may be varied according to needs. Materials are not of critical importance either, unless highly corrosive wastes will be encountered. In our case, availability of materials and the ease with which they could be

worked were the chief factors considered. Two-inch lumber and large nails are used to construct load-bearing structures (sides, ends, and cross bracing), while the pipe supports and grate-retaining rim are of 1-inch material. All wooden parts were given two coats of high-grade enamel. The sections of aluminum grating which hold the jars were part of the covering for the floor trench over which the jar-rinsing rack is supported and into which waste water drains. Wooden cross-pieces could be substituted for the metal gratings at considerable savings in material costs. Plumbing components include 3/4-inch plastic pipe and fittings (1/2-inch pipe would be adequate for a small rack), 3/4-inch valves (one for each pipe), 1/4-inch brass spray nozzles, and 1/4- by 1 1/8-inch all-thread nipples for attaching nozzles to the pipe. Total cost of materials exclusive of the gratings was \$54.20.

USE

After the jars are emptied and removed from bioassay troughs, they are cleaned according to a procedure like that outlined by Lennon and Walker (1964). The rack not only provides jar support at a convenient working height during washing, but also cuts the time for washing and rinsing to less than half that required when jars were cleaned individually.

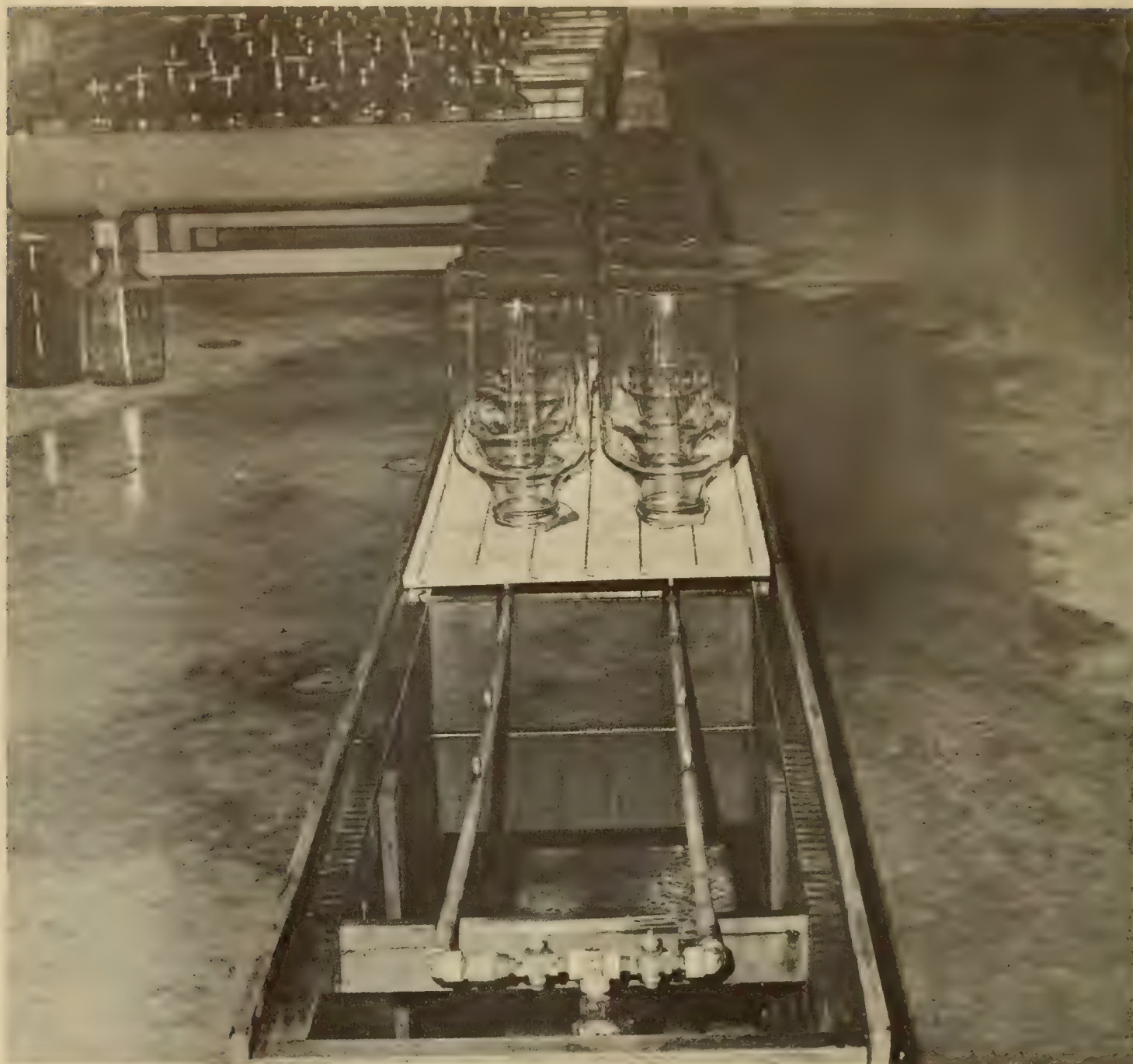


Figure 1.--Jar-rinsing rack with sections of grating removed to show construction detail.

When fewer than 26 jars are rinsed, a board is laid over the unused nozzles to deflect water into the floor trench.

AUTOMATIC LIQUID MEASURING VESSEL

DESCRIPTION

Most of the custom-made shell of the vessel was constructed of sections of polyethylene pipe having a wall thickness of one-fourth inch and inside diameters of 3, 4, and 8 inches

(fig. 2). The main body of the vessel was made by heat-fusing a specially shaped top and bottom on a 17-inch-long section of 8-inch pipe. The convex top plate was made from a polyethylene block machined to a thickness of one-fourth inch, and the weight-bearing, concave lower plate was similarly machined to a thickness of one-half inch. An 8-inch-long piece of the 4-inch pipe was fused to the top plate to form the neck of the vessel, and a 1 1/2-inch-long piece of 3-inch pipe was fused to the bottom plate to serve as a drain (it also holds the vessel in place on the jar).

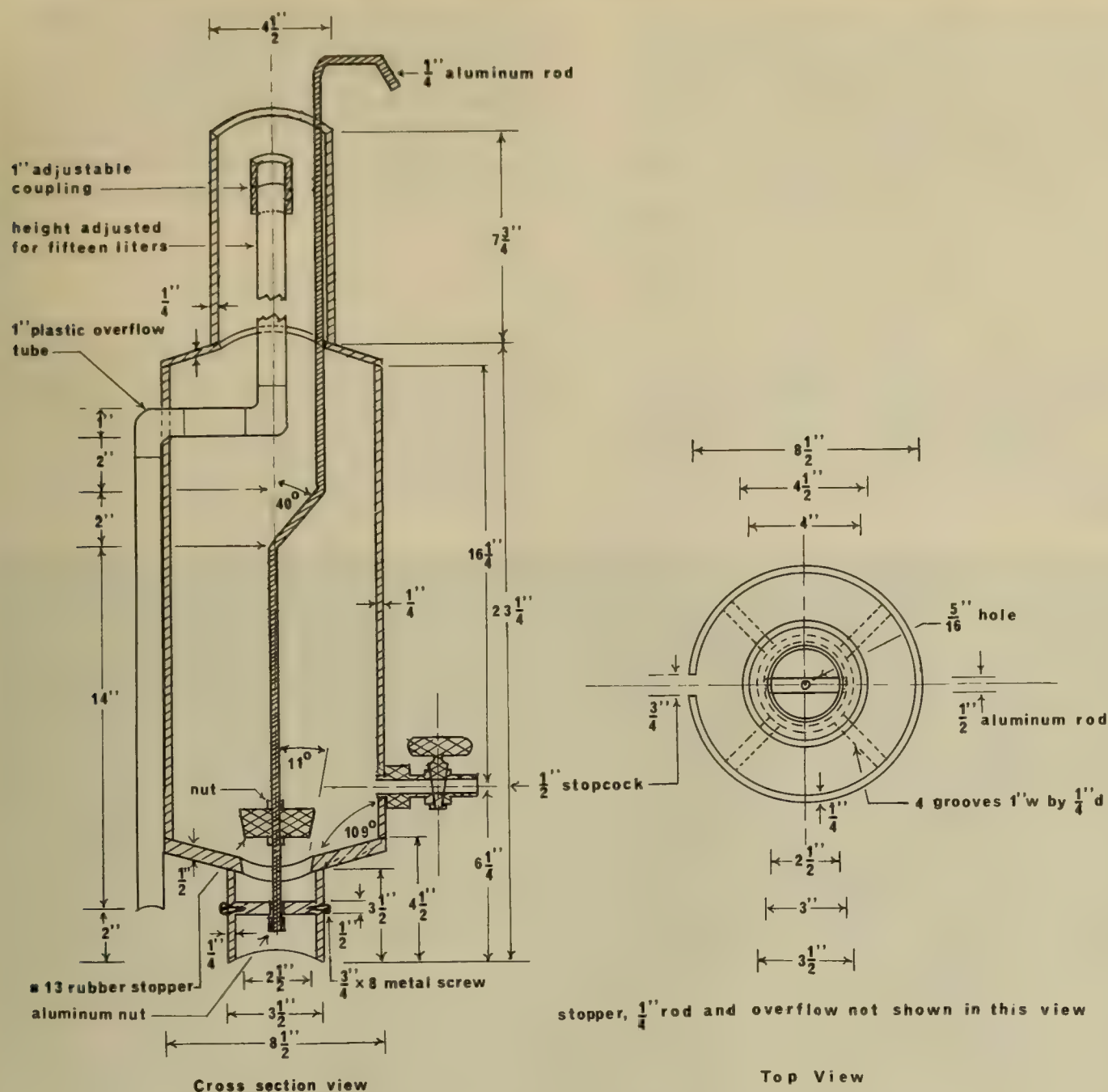


Figure 2.--Schematic illustration of automatic measuring vessel.

Special shaping of the upper and lower plates prevents air bubbles from being trapped under the upper plate, and results in rapid, complete drainage from the lower plate. The underside of the lower plate was made with four radial grooves one-half inch wide and one-fourth inch deep to permit rapid escape of air from the jar as it fills.

In filling the vessel, water enters through a $\frac{1}{2}$ -inch hard-rubber stopcock near the bottom, and any excess overflows through a 1-inch plastic pipe inserted through the side of the vessel near the top. The internal upper

section of the overflow pipe extends upward into the neck to the required level, and the external lower section extends down to the bottom of the vessel where it is rigidly attached. A threaded plastic coupling at the top of the overflow allows for calibration of the volume measured.

The vessel is emptied through a drain hole in the bottom plate, the sides of which are tapered at an angle of 11° . A No. 13 rubber stopper and a 34-inch-long piece of aluminum rod serve as valve and handle, respectively. The lower 6 inches of the rod are threaded.

The stopper, which is located about 5 inches from the lower end of the rod, is held securely in place by means of aluminum washers and nuts above and below it. The lower end of the rod extends through a hole in a 3-inch-long piece of 1/2-inch aluminum rod which is held in place across the inside of the drain by small metal screws in each end. This guides the stopper into place, and when the vessel is emptied, upward travel of the rod is limited by the aluminum nut on its lower end. The midsection of the handle is bent to form a 2-inch offset which gives clearance around the upper end of the overflow pipe. The upper end of the rod extends slightly above the top of the vessel and is bent at a right angle to form a hand grip.

In the last vessel constructed, a stainless-steel rod was used for the handle, and a toilet-tank ball of soft, flexible rubber was used for a valve. The dimensions of the vessel could be changed easily to permit measurement of a wide range of volumes. The total cost of materials for the vessel was \$37.20.

USE

To facilitate rapid filling of jars, two vessels are used concurrently. Water is supplied to each through sections of 5/8-inch plastic tubing connected to a single hose by a "Y" connector (fig. 3). Under our conditions it takes about 25 seconds to fill the measuring

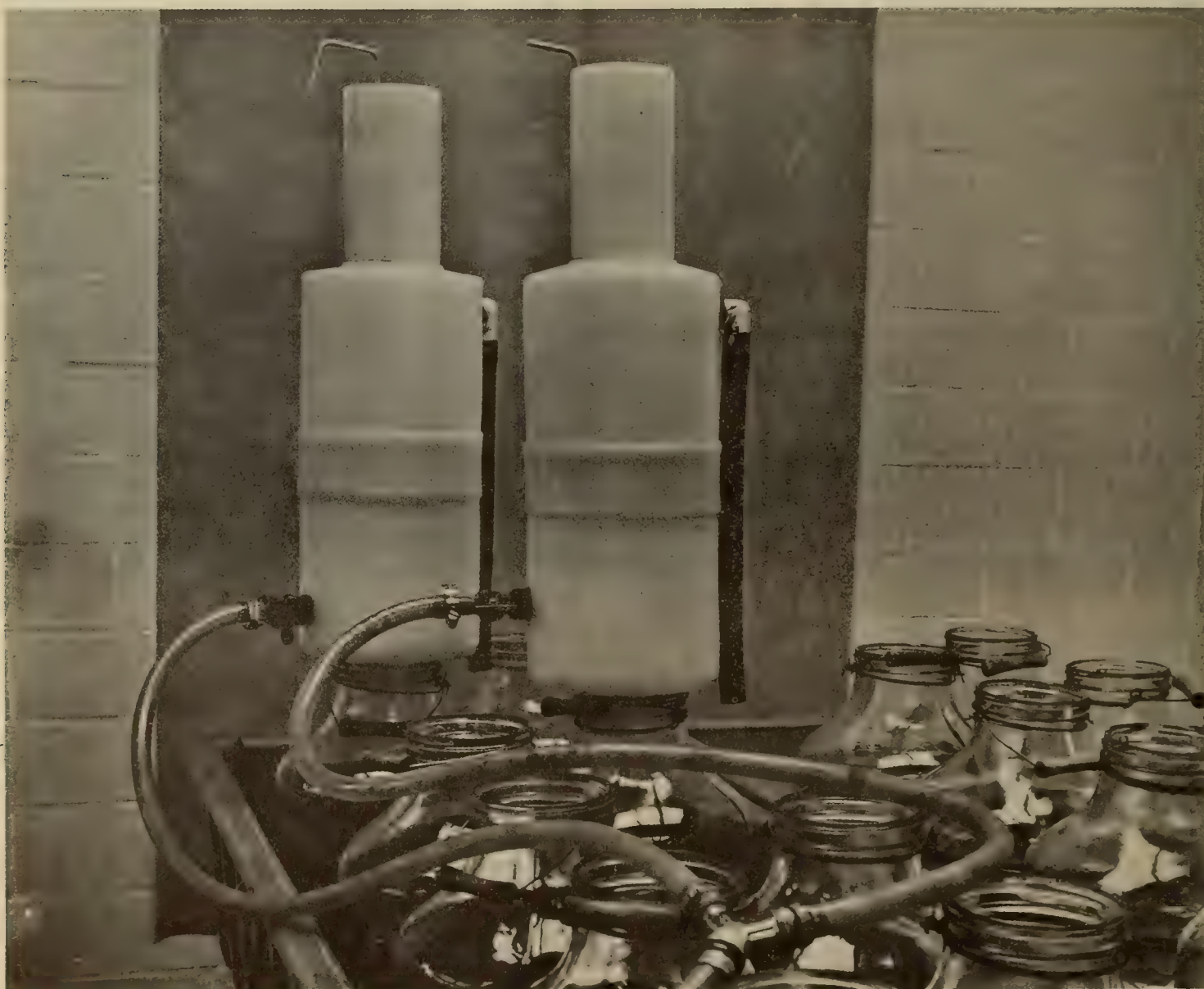


Figure 3.--Automatic measuring vessels ready for use.

vessel with 15 liters of water, and less than half that long to empty it. Thus, a full vessel can be emptied and moved to another jar while the other vessel is filling. The plastic vessels are light, safe to handle, and quite durable.

JAR EMPTIER

DESCRIPTION

This tool consists of a U-shaped tube made of 1 1/2-inch plastic pipe, about a foot of which is inserted through a rubber seal (fig. 4). The seal is fashioned from the thick-walled upper section of a 2-way force cup

(plumber's helper). A short section of 3/8-inch cooper tubing having a flared inner end also is inserted through the rubber cup to pass compressed air into the jar. The intake end of the 1 1/2-inch pipe is covered with a 1/8-inch mesh screen to prevent loss of fish as water is removed. The cost of materials was \$2.35.

USE

The safe, low-pressure (5 p.s.i.) air supply used for aerating fish tanks provides the power to empty water from the bioassay jars. Once the screened end of the jar emptier and the rubber seal are inserted in the jar, compressed air seats the seal firmly against the



Figure 4.--Jar emptier partially inserted into jar; compressed air is supplied through the small tube while waste water is expelled through the 1 1/2-inch pipe.

neck. Nearly 15 liters of water are expelled in 11 seconds, and the resultant 30-pound reduction in weight makes the task of lifting jars from bioassay troughs much easier and safer. Tests were conducted to learn what would happen when the outlet screen was deliberately plugged and pressure was allowed to build up. In each case the seal was harmlessly forced out the neck of the jar, demon-

strating that the necessary margin of safety was provided.

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18. Toxicity of 22 Therapeutic Compounds to Six Fishes, by Wayne A. Willford. (Resource Publication 35.) 1967. 10p.
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INVESTIGATIONS IN FISH CONTROL

22. Efficacy of Quinaldine as an Anesthetic
for Seven Species of Fish
23. Toxicity of Quinaldine
to Selected Fishes
24. Quinaldine as an Anesthetic
for Brook Trout, Lake Trout,
and Atlantic Salmon

APR 10 1969



United States Department of the Interior
Fish and Wildlife Service
Bureau of Sport Fisheries and Wildlife

INVESTIGATIONS IN FISH CONTROL

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16. Annotated Bibliography on MS-222, by Richard A. Schoettger. (Resource Publication 22.) 1967. 15p.
17. MS-222 as an Anesthetic for Channel Catfish: Its Toxicity, Efficacy, and Muscle Residues, by Richard A. Schoettger, Charles R. Walker, Leif L. Marking, and Arnold M. Julin. (Resource Publication 33.) 1967. 14p.

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Continued on inside back cover--

INVESTIGATIONS IN FISH CONTROL

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By Leif L. Marking, Chemist

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Lake Trout, and Atlantic Salmon

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ERRATA

<u>Paper</u>	<u>Page</u>	<u>Column</u>	<u>Par.</u>	<u>Line</u>	<u>Correction</u>
22	4	2	3	9	IN sodium hydroxide
22	5	2	2	4	Schoettger and Julin (1967)
22	6	2	3	9	non-ionic to an ionic form
22	7	-	Table 2		(Stage II)
22	8	1	Table 3		(Stage II)
23	3	1	2	8	Schoettger and Julin (1969)
23	4	2	1	-	7.5 milliliters. 250 milliliters.
23	9	1	2	8	Schoettger and Julin (1969)
23	9	1	2	10	pH 5
23	10	2	-	-	Schoettger, Richard A., and Arnold M. Julin 1969. Investigations in Fish Control 22. The efficacy of quinaldine as an anesthetic for seven species of fish. U.S. Bureau of Sport Fisheries and Wildlife, 10 p.
24	3	2	2	2	37.85 milliliters. 40 milliliters.
24	5	2	References		Delete: Bové, Frank J. Meister, Alfred L., and Charles F. Ritzi Stecher, Paul G.

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UNITED STATES DEPARTMENT OF THE INTERIOR
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EFFICACY OF QUINALDINE AS AN ANESTHETIC FOR SEVEN SPECIES OF FISH

By Richard A. Schoettger and Arnold M. Julin, Fishery Biologists
Bureau of Sport Fisheries and Wildlife
Fish Control Laboratory, La Crosse, Wisconsin

ABSTRACT.--Quinaldine was tested as an anesthetic for rainbow trout, brown trout, brook trout, lake trout, channel catfish, bluegill, and largemouth bass. In general, 15 to 70 ppm of the drug induce total loss of equilibrium in fish within two minutes. Efficacy is influenced by acid pH and for some species, by temperature, but not by water hardness, age of quinaldine solutions, or repeated exposures of fish to quinaldine. Assets include rapid action and prolonged maintenance of anesthesia, but anesthetized fish retain a degree of reflex responsiveness which may interfere with stripping, delicate surgical operations, and blood collection. The drug is harmless to fertilized rainbow trout eggs at concentrations and exposure times normally encountered in spawning operations.

Quinaldine (2-methylquinoline) is obtained from coal tar and is used in the manufacture of dyes (Turner, 1950). Its anesthetic effect on fish was first reported by Muench (1958), who found that concentrations of 2.5 to 20 ppm narcotized goldfish, golden shiners, yellow bullheads, green sunfish, and white crappies within 0.5 to 4 minutes. The relatively small number of published reports on the use of quinaldine as a fish anesthetic, in contrast to MS-222 (Schoettger, 1967), suggests that it is not widely used by fishery workers. However, in our survey of chemicals used at national fish hatcheries, we found that it is employed in handling a variety of species including sockeye salmon, chinook salmon, coho salmon, rainbow trout, brown trout, brook trout, northern pike, goldfish, channel catfish, smallmouth bass, largemouth bass, and walleye. Natarajan and Ranganathan (1960) used quinaldine in fish transport, and Greenough (1963) patented a fish-transport medium containing a buffer, an antibiotic, and quinaldine. Penfold (1965) found the anesthetic useful in the live collection of certain marine species.

Recent amendments to the Federal Food, Drug, and Cosmetic Act require that chemicals used on fish be cleared and labeled for their specific uses (Lennon, 1967). The information needed to clear quinaldine includes its toxicity to fish, its efficacy as an anesthetic, its residues in fish tissues, and its safety to other animals. The intent of our research was to extend Muench's (1958) investigations on efficacy to include species more commonly cultured in hatcheries, such as fingerling and adult trout, catfish, and centrarchids. Also, we wished to study the influence of temperature, water quality, and repeated narcosis of fish on the efficacy and stability of quinaldine solutions, and the toxicity of quinaldine to trout eggs.

Spector (1956) cited an oral LD₅₀ for rats of 1.23 g./kg. and a cutaneous toxicity to rabbits of 1.87 g./kg. Both values classify quinaldine as slightly toxic to mammals. Bell (1964) recommended avoidance of vapor inhalation and prolonged skin and eye contact. According to personal communication from Dr. Hans L. Falk, Associate Director for

Carcinogenesis, National Cancer Institute, Department of Health, Education and Welfare, Bethesda, Maryland, November 10, 1966, there is no evidence that quinaldine has carcinogenic properties, either on the basis of bioassay, or on correlation with structural requirements for carcinogenicity. Quinaldine N-oxide, the only related chemical they tested for carcinogenicity, was inactive.

METHODS AND MATERIALS

Quinaldine used in these investigations was 95 percent technical material purchased from Eastman Kodak Company. Efficacy of the chemical was tested with rainbow trout, brown trout, brook trout, lake trout, channel catfish, and largemouth bass of two to six inches and seven to 12 inches (table 1). Bluegills of two to six inches were also tested. The trials with trout were conducted at 7°, 12°, and 17° C., and those with channel catfish and centrarchids at 7°, 12°, 17°, 22°, and 27° C.

The methods of preparing test solutions and various water qualities, acclimating the test fish, and evaluating efficacy were essentially the same as those described by Schoettger and Julin (1967), with some modifications. The major modifications were in the preparation of stock solutions and in the criteria for efficacy. Stock solutions were prepared by diluting the

compound with acetone and water. Sufficient acetone was added to produce clear solutions.

The changes in criteria for efficacy were related to the differences in behavioral responses of quinaldine-treated and MS-222-treated fish. The responses of the former are discussed later. Concentrations inducing total loss of equilibrium (stage 11) within 2 minutes were considered effective. Following application of anesthesia, the fish were held in the test solutions until they entered medullary collapse, or for 6 hours, whichever occurred first. This period gave a measure of the tolerated exposure time. The fish were then placed in flowing well water for recovery.

During the investigations, we observed that pH influenced the effectiveness of quinaldine. Additional trials were carried out to better define this influence and to determine whether it was reversible with changes in pH. Smallmouth bass were tested in solutions containing 20 and 30 ppm of quinaldine; channel catfish in 50 and 60 ppm. The pH of solutions was manipulated with IN sodium hydroxide and 0.25M phthalic acid. The test solutions were maintained at 12° C. in water baths.

The influence of water hardness on the efficacy of quinaldine was measured with 2.5-inch rainbow trout at 12° C. Solutions of 10 and

Table 1.--Species and sources of fish

Common name	Scientific name	Source
Rainbow trout....	<u>Salmo gairdneri</u>	NFH, ^{1/} Manchester, Iowa
Brown trout.....	<u>Salmo trutta</u>	SFH, ^{2/} Lanesboro, Minn.
Brook trout.....	<u>Salvelinus fontinalis</u>	SFH, Lanesboro, Minn.
		SFH, Osceola, Wis.
Lake trout.....	<u>Salvelinus namayacush</u>	NFH, Jordan River, Charlevoix, Mich.
		SFH, St. Croix Falls, Wis.
Channel catfish..	<u>Ictalurus punctatus</u>	NFH, Fairport, Iowa
		NFH, Guttenberg, Iowa
		SFH, Lansing, Iowa
Bluegill.....	<u>Lepomis macrochirus</u>	NFH, Guttenberg, Iowa
		NFH, Lake Mills, Wis.
Smallmouth bass..	<u>Micropterus dolomieu</u>	NFH, Fairport, Iowa
Largemouth bass..	<u>Micropterus salmoides</u>	NFH, Genoa, Wis.

^{1/} National Fish Hatchery

^{2/} State Fish Hatchery

180 ppm of total hardness were prepared as described by Schoettger and Julin (1967) and contained 15 ppm of quinaldine. Ten fish were used to bioassay each test solution. Effects on efficacy were judged by the times to induce total loss of equilibrium (stage 11) in all fish.

The effect of repeated exposures to quinaldine was tested by treating ten 3.6-inch rainbow trout daily in a concentration of 12 ppm at 12° C. The times to total loss of equilibrium (stage 11) and recovery, and the percent survival were used as indexes of sensitivity. After six consecutive treatments, the susceptibility of the fish was compared with that of untreated controls.

The potency of quinaldine solutions aged up to 50 days at 12° or 27° C. were determined by bioassay. The tests at 12° were conducted in polyethylene tanks containing 45 liters of a 15-ppm solution. Temperature was maintained within $\pm 2^\circ$ C. by water baths, and at intervals efficacy was checked against ten 5-inch rainbow trout. Two-inch bluegills were used in bioassays at 27° since trout are difficult to maintain at this temperature. A level of 12 ppm was tested at 27° because, at the time, effective concentrations had not yet been established for bluegills. The volume of some solutions at both temperatures was reduced during aging by evaporation, but oxygen levels were not seriously affected. Water losses were replaced with deionized water before the tests.

The quantity of fish which can be anesthetized per milliliter of quinaldine was estimated by narcotizing a number of 5-inch rainbow trout in the same solution until it became ineffective. The test was conducted in 2.5 liters of a 15-ppm solution which was aerated and maintained at 12° C. Five individuals at a time were anesthetized to total loss of equilibrium (stage 11) removed, weighed, and placed in fresh water. None of the fish was exposed to the chemical more than once. The test solution was considered ineffective when, in consecutive trials, fewer fish were anesthetized.

The toxicity of quinaldine to fertilized eggs of rainbow trout was determined at concentrations of 15, 30, 60, and 120 ppm. Sixty to

70 eggs, 24 hours old, were placed in 2.5 liters of each concentration. Fifteen eggs were removed from each solution after 15, 30, 60, and 120 minutes of exposure, rinsed, placed in petri dishes, and incubated in well water at 12° C. Four groups of control eggs were placed in re-constituted water without quinaldine and then incubated like the treated eggs. Mortalities among the quinaldine-treated and control eggs were recorded 96 hours after treatment, and thereafter at intervals until hatching.

RESULTS AND DISCUSSION

BEHAVIOR OF ANESTHETIZED FISH

The behavior of quinaldine-treated fish is different, in some respects, from that of fish exposed to other anesthetics (McFarland, 1960; Schoettger and Julin, 1966). At first, the chemical causes irritation which seems to increase in intensity with concentration. Shortly thereafter they lose equilibrium without entering a pronounced stage of sedation. Anesthesia progresses rapidly to total loss of equilibrium (stage 11) and then slows. At this level the fish are relatively motionless and may rest upright, inverted, or on their sides on the bottom of the container. They can be handled gently, but striking the container or squeezing the caudal peduncle or fin induces strong reflex movements. Bell (1964) indicated that quinaldine was useful for surgical operations on coho salmon, but in operations on rainbow trout we observed periodic reflex movements that hindered surgery.

Fish can be maintained in total loss of equilibrium, (stage 11) for relatively long periods, depending on concentration, before the onset of loss of reflex and medullary collapse. The loss of reflex stage appears to be practically nonexistent. Thus, loss of equilibrium is best suited for evaluating the efficacy of quinaldine.

The mode of action of quinaldine in fish is unknown. Bell (1964) suggested that it may act like the barbiturates as depressants on the central nervous system and especially the respiratory center. We measured opercular rates in anesthetized and control rainbow trout. The rate in the latter was approximately 60 per minute. In treated individuals the rate increased

to more than double the control value after 5 minutes of exposure. Although the rate in treated fish was much faster, the opercular movements appeared weak and were interrupted by periodic gasps.

EFFICACY

Concentrations of 15 or 16 ppm were, in most instances, at least 90 percent effective for inducing anesthesia within 2 minutes in rainbow trout, brown trout, brook trout, and lake trout (table 2). The mean exposures tolerated by the trout ranged from 45 minutes to more than 6 hours. Most individuals recovered in fresh water within several minutes, but some required over 60 minutes. Temperature and size of trout appear to have no influence on efficacy, or on exposure and recovery time.

As many as 25 percent of the test fish died in some trials (table 2). This was not delayed mortality, but resulted from attempts to measure the longest exposure tolerated by each test group. Since the fish could not be observed constantly during the experiments, the most susceptible individuals were overexposed. The procedure may have contributed to the long recovery times noted in some trials. However, the mortalities of trout should be minimal when the progress of anesthesia can be watched more closely.

Channel catfish, bluegills, and largemouth bass are more resistant at temperatures of 17° C. and lower than are salmonids (table 2). Catfish were anesthetized by 70 ppm, bluegill by 15 to 60 ppm, and bass by 20 to 70 ppm. At 22° or 27° C., about 15 ppm were effective on bluegill and bass, and 30 ppm narcotized catfish. The results show that 7- to 12-inch bass are much more resistant to quinaldine than smaller individuals.

The relatively high concentrations required to anesthetize catfish, bluegills, and bass at 17° C. and below shortened the mean exposure and lengthened recovery time (table 2). In general, the fish tolerated exposure for about 5 to 20 minutes at levels exceeding 15 ppm. At lower concentrations they commonly tolerated exposures of one to more than six hours.

Recovery time appeared to be more related to temperature and concentration than to exposure time, especially in bluegills and largemouth bass. For example, bluegills recovered in 2 to 4 minutes after a 6-hour exposure to 15 ppm at 27° C., whereas more than 60 minutes were required at 7° after a 0.4-hour exposure to 60 ppm. This may indicate an effect of low temperature on the excretion or metabolic deactivation of quinaldine.

Effects of pH.--The efficacy trials shown in table 2 were carried out at pH 7.0 and 8.5. We combined the data since there appeared to be no difference in the results at these pH values. The trials at pH 5.0 gave quite different results. The drug was completely ineffective on all seven species. Further trials were carried out to determine the approximate degree of acidity which deactivated quinaldine. Nine smallmouth bass were anesthetized in a 20-ppm solution at 12° C. and pH 7.0. The pH was changed to 5.0 and the fish recovered in 20 to 25 minutes. In another experiment the pH was changed to 5.7, but the fish did not recover within a 2-hour period. Thus, a pH of about 6.0 or above does not deactivate quinaldine.

Acidic solutions apparently do not destroy quinaldine. Channel catfish were not affected by a 1-hour exposure to either 50 or 60 ppm of the chemical at pH 5.0. When the pH of the former solution was raised to 7.0, and that of the latter to 10.3, the fish were narcotized in 3 to 10 minutes. This suggests that under acid conditions the quinaldine molecule shifts from an ionic to a non-ionic form which is less biologically active.

Effects of water hardness.--Quinaldine was as effective on rainbow trout in soft water (10 ppm total hardness) as in hard water (180 ppm total hardness). Four trials were run in soft water and two in hard water, and in each case all of the fish were anesthetized within 2 minutes.

Effects of repeated anesthetization.--Daily exposure of rainbow trout to 12 ppm of quinaldine for 6 days did not influence their sensitivity to the drug. The fish were anesthetized within 1 to 3.5 minutes, and variations in efficacy appeared random. They were exposed to the chemical for

Table 2.--Concentrations of quinaldine which anesthetize seven species of fish to total loss of equilibrium (stage 11) within 2 minutes

Species	Concentration (ppm)	Temperature (° C.)	Fish		Fish in anesthesia		Mean Exposure Time (hrs.)	Safe Exposure Index ^{1/}	Recovery	
			Size (in.)	Number tested	(number)	(percent)			Mean time range (min.)	Survival (percent)
Rainbow										
trout...	15	7°	2- 6	45	43	96	0.8	24	6-13	100
Do.....	15	7°	7-12	20	19	95	6.0	180	-	95
Do.....	15	12°	7-12	20	19	95	2.3 ^{2/}	-	1-4	100
Do.....	15	12°	7-12	20	20	100	6.0	180	-	100
Do.....	15	17°	7-12	25	16	64	6.0	180	3- 5	76
Do.....	16	17°	7-12	25	25	100	3.0	90	5-43	76
Brown										
trout...	16	7°	7-12	20	20	100	6.0	180	13-23	100
Do.....	16	12°	2- 6	10	10	100	>6.0	>180	-	10
Do.....	16	12°	7-12	15	15	100	6.0	180	5- 8	100
Do.....	16	12°	7-12	5	5	100	4.0	120	<25	100
Do.....	16	17°	7-12	20	20	100	6.0	180	4- 6	100
Brook										
trout...	16	7°	2- 6	20	20	100	1.7	51	3-21	80
Do.....	16	7°	7-12	20	20	100	6.0	180	15-27	95
Do.....	16	12°	2- 6	40	35	88	1.0	30	3->60	88
Do.....	16	12°	7-12	20	20	100	0.5 ^{2/}	-	2- 4	100
Do.....	16	17°	7-12	20	20	100	6.0	180	-	95
Lake										
trout...	15	7°	2- 6	40	35	88	1.0	30	5-10	75
Do.....	16	7°	2- 6	30	30	100	1.8	54	10->60	90
Do.....	15	7°	7-12	24	24	100	6.0	180	<40	92
Do.....	15	12°	2- 6	100	100	100	2.9	57	2-15	98
Do.....	15	12°	7-12	20	20	100	6.0	180	<30	90
Do.....	15	17°	7-12	24	20	83	6.0	180	6- 9	100
Channel										
catfish.	70	7°	7-12	42	42	100	0.1	3	11-24	100
Do.....	70	12°	2- 6	70	70	100	0.1 ^{2/}	-	5-11	100
Do.....	70	12°	7-12	30	30	100	0.1	3	5- 6	100
Do.....	70	17°	2- 6	70	70	100	0.1 ^{2/}	-	4- 7	100
Do.....	70	17°	7-12	10	10	100	0.1	3	3- 6	100
Do.....	30	27°	2- 6	10	10	100	1.0	30	3- 4	80
Do.....	30	27°	7-12	5	4	80	6.0	180	<5	100
Do.....	30	27°	7-12	10	10	100	1.2	36	1- 3	100
Do.....	30	27°	7-12	5	5	100	>6.0	>180	1	100
Bluegill..										
Do.....	60	7°	2- 6	37	29	78	0.4	12	>60	92
Do.....	30	12°	2- 6	30	28	93	0.3	9	12->60	100
Do.....	15	17°	2- 6	20	20	100	6.0	180	4- 8	100
Do.....	10	22°	2- 6	20	20	100	0.3	9	20-35	100
Do.....	15	27°	2- 6	30	30	100	1.0	30	2-20	93
Do.....	15	27°	2- 6	10	10	100	6.0	180	2- 4	100
Largemouth										
bass....	30	7°	2- 6	20	19	95	1.8	54	36->60	95
Do.....	70	7°	7-12	5	5	100	0.1	3	40->60	100
Do.....	15	12°	2- 6	60	59	98	1.8	54	10-33	92
Do.....	30	12°	7-12	70	70	100	0.1 ^{2/}	-	8-13	100
Do.....	20	17°	7-12	21	19	90	0.4 ^{2/}	-	6-29	95
Do.....	16	22°	2- 6	20	20	100	2.5	75	2- 3	95
Do.....	15	27°	2- 6	40	39	98	4.5	135	1- 2	100

1/ Index obtained by dividing the time for the first fish to reach medullary collapse by the time (2 minutes) for fish to reach total loss of equilibrium, stage 11.

2/ Fish removed from the anesthetic before reaching medullary collapse.

periods of approximately 2 to 20 minutes, and all recovered in fresh water within 2 minutes. The response of fish receiving six treatments was essentially the same as those not treated previously.

Effects of aging.--The aging of quinaldine solutions at 12° C. appears to have little effect on efficacy. Concentrations of 15 ppm which were aged for 50 days anesthetized rainbow trout within 2.0 minutes. This compares favorably with the effectiveness of solutions bio-assayed immediately after preparation. At 27° C. quinaldine was less effective on bluegill after the solutions were aged for 21 days (table 3). These data show that solutions of the anesthetic should be usable over relatively long periods, unless fouled by mucous or excrement, but they may require aeration. We found that aeration for as long as 24 hours does not diminish efficacy.

Table 3.--Influence of aging on efficacy of 12 ppm of quinaldine for bluegills at 27° C.

Solution age (days)	Number of fish	Fish in loss of equilibrium stage 11		Recovery in fresh water (number)
		Num-ber	Time (Min.)	
0	10	10	1.0-2.0	10
1	10	10	0.5-1.5	10
3	10	10	1.0-2.0	10
7	10	10	0.5-1.0	10
14	10	10	0.5-2.5	10
21	10	0	-	10
21	10	4	7.0-9.0	10

Repeated use of solutions.--The approximate "life expectancy" of quinaldine solutions was determined by anesthetizing groups of rainbow trout in a concentration of 15 ppm. A total of 3,542 grams of fish were treated in a solution containing 0.037 ml. of quinaldine before the exposure required for anesthesia exceeded 2 minutes. This ratio of fish weight to quinaldine amounts to 94.5 kg./ml. Meister and Ritzi (1958) made similar measurements for MS-222. They found that about 14 kg. of brook trout and 42 kg. of lake trout could be effectively narcotized per gram of drug.

TOXICITY TO EGGS

Fifteen- to 30-minute exposures of fertilized rainbow trout eggs to concentrations as high as 120 ppm of quinaldine were apparently not detrimental (table 4). Two-hour exposures to concentrations of 60 and 120 ppm killed approximately 40 and 100 percent of the eggs respectively within 4 days, while mortalities at 15 and 30 ppm were relatively light. Some random mortalities occurred in the treated and control eggs throughout the balance of the incubation period. The remaining eggs hatched approximately 21 days later. Since most spawning operations would probably be carried out with concentrations of 15 to 30 ppm, and since fertilized eggs are placed subsequently in flowing water, it is unlikely that accidental contamination of eggs would be detrimental.

Table 4.--The toxicity of quinaldine to fertilized, rainbow trout eggs at 12° C.

Concentration (ppm)	Number of eggs	Number of dead eggs within 4 days after exposure to quinaldine for			
		0.25 hours	0.50 hours	1.00 hours	2.00 hours
Control	60	0	0	0	0
15	15	0	0	0	1
30	15	0	0	0	1
60	15	0	0	2	6
120	15	0	0	1	15

METABOLISM OF QUINALDINE

The metabolism of quinaldine in fish has not been elucidated. Early investigations with other animals indicated that the chemical may be converted into natural metabolites. Kusui (1931) concluded that frogs oxidized a small portion of subcutaneously injected quinaldine to quinaldic acid which was excreted in the urine. Takahashi (1931) reported that chickens and rabbits transformed the compound into alpha-quinolinic acid, some of which was conjugated with glycine. Later studies have shown that relatively large amounts of 4-methylquinoline

(lepidin) are oxidized by chickens, but only trace quantities of 2-methylquinoline (quinaldine) are changed to quinaldic acid (Tsunoo et al., 1965). Quinaldic acid is a normal metabolite of tryptophan in various mammals and may be conjugated with glycine and excreted in the urine (Roy and Price, 1959; Kaihara, 1960; Kaihara and Price, 1961). Thus, even in homotherms, the metabolic fate of quinaldine is not well understood.

SUMMARY

Concentrations of 15 or 16 ppm of quinaldine rapidly induce total loss of equilibrium in trout. Fifteen to 30 ppm are effective on channel catfish, bluegills, and largemouth bass at temperatures of 22° and 27° C. At lower temperatures, concentrations up to 60 or 70 ppm are needed to achieve rapid anesthesia in the largemouth bass. Quinaldine solutions with a pH of 5.0 failed to anesthetize fish, but efficacy was restored by increasing basicity. Water hardness, age of solutions, and repeated exposures of fish to quinaldine appear to have little influence on efficacy. The chemical is toxic to fertilized rainbow trout eggs only after several hours exposure to relatively high concentrations.

The utility of quinaldine as a fish anesthetic depends in large part on the needs of the fishery worker. Its major assets include rapid action and prolonged maintenance of anesthesia. On the other hand, anesthetized fish retain a degree of reflex responsiveness which may interfere with stripping, delicate surgical operations, and blood collection.

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INVESTIGATIONS IN FISH CONTROL

**23. Toxicity of Quinaldine
to Selected Fishes**

By Leif L. Marking, Chemist



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TOXICITY OF QUINALDINE TO SELECTED FISHES

By Leif L. Marking, Chemist
Bureau of Sport Fisheries and Wildlife
Fish Control Laboratory, La Crosse, Wisconsin

ABSTRACT.--Quinaldine, an anesthetic for fish, is toxic to various sizes of rainbow trout, brown trout, brook trout, lake trout, northern pike, channel catfish, bluegills, largemouth bass, and walleyes in 15-, 30-, and 60-minute and 3-, 6-, 24-, 48-, and 96-hour static bioassays. Toxic concentrations range from 2.0 to 25 ppm in standard tests at 12° C. in 96 hours. Its toxicity to rainbow trout is significantly greater at higher temperatures, and 96-hour LC50's range from 13.3 ppm at 7° to 1.9 ppm at 17° C. In 6-hour exposures, quinaldine is more toxic at colder temperatures. Bluegills are more sensitive at 22° than at 12° or 17° C. The anesthetic is more toxic to fish in hard than in soft water, a condition probably associated with pH. Safety indexes show that shorter exposures to quinaldine are safer to fish, although the concentrations may be greater than required in longer exposures. Recovery from anesthesia is good among survivors in fish exposed to partial-kill concentrations of quinaldine for 96 hours.

Anesthetics are rapidly becoming more important and widely used in fisheries. Varieties of anesthetics have been found useful in marking, tagging, transporting, and spawning fish of various species (Parkhurst and Smith, 1957; McFarland, 1960; Bell, 1964). Practical concentrations of chemicals to produce desirable anesthesia in fish have been defined under field conditions by several workers (Meister and Ritzi, 1958; Thompson, 1959). Klontz (1964) outlined 14 methods used to anesthetize fish.

Quinaldine was reported to be an anesthetic for fish by Muench (1958). More recently, Matarajan and Ranganathan (1960) and Greenough (1963) discussed the usefulness of quinaldine in fisheries. A comprehensive study of the compound's efficacy as an anesthetic for seven freshwater fish species was made by Schoettger and Julin (1968).

Quinaldine (2-methylquinoline) occurs in coal tar. It can be manufactured by treatment of aniline and paraldehyde with hydrochloric acid and heat (Rose and Rose, 1966) or derived from aniline, acetaldehyde, and hydrochloric acid (Stecher et al., 1960). Quinaldine boils at 246° - 247° C., darkens from light yellow to brown with exposure to air, is soluble in alcohol, ether, chloroform, and acetone, but is insoluble in water (Rose and Rose, 1966).

This study was undertaken to establish the toxicity of practical-grade quinaldine to nine species of fish.

METHODS AND MATERIALS

Nine species of fish were obtained from fish hatcheries (table 1). Three size groups included 1- to 3-inch, 3- to 5-inch, and 6- to 9-inch fish.

Table 1.--Fishes used in tests of quinaldine

Common name	Scientific name	Source
Rainbow trout....	<u>Salmo gairdneri</u>	NFH, Manchester, Iowa
Brown trout.....	<u>Salmo trutta</u>	NFH, Manchester, Iowa
Brook trout.....	<u>Salvelinus fontinalis</u>	SFH, Osceola, Wis.
Lake trout.....	<u>Salvelinus namaycush</u>	NFH, Jordan River, Mich. SFH, St. Croix Falls, Wis.
Northern pike....	<u>Esox lucius</u>	NFH, Garrison Dam, N. D. NFH, Gavins Point, S. D.
Channel catfish..	<u>Ictalurus punctatus</u>	NFH, Fairport, Iowa
Bluegill.....	<u>Lepomis macrochirus</u>	NFH, Lake Mills, Wis.
Largemouth bass..	<u>Micropterus salmoides</u>	NFH, Genoa, Wis.
Walleye.....	<u>Stizostedion vitreum</u>	NFH, Garrison Dam, N. D.

Ten fish were included at each of 10 or 11 concentrations of quinaldine in 15-liter, static bioassays as described by Lennon and Walker (1964). Ten to twenty of the 1- to 3-inch fish served as controls depending on how many concentrations were tested. The bioassays with 3- to 5-inch and 6- to 9-inch fish were made in polyethylene tanks containing 45 liters of aerated solution. Five concentrations were tested against the 9-inch fish, and 10 fish served as controls.

Variations in water quality were arranged by adding different amounts of reconstituting salts to deionized water (table 2). Temperatures of 7°, 12°, 17°, and 22° C. were maintained by placing the bioassay vessels in thermostatically controlled water baths. All temperatures listed hereafter are in Centigrade.

Concentrated stock solutions of the practical-grade quinaldine manufactured by Eastman Organic Chemicals were mixed daily to insure complete activity and prevent degrada-

tion. Acetone was used to dissolve 7.5 milliliter of quinaldine and this was diluted with deionized water. Approximately 50 percent of the final 250 milliliter of stock volume was water.

Fish responses to quinaldine were recorded for several hours after exposure and daily thereafter throughout the 96-hour bioassay. Dead fish were recorded and removed. Live fish were readily distinguishable because they were hypersensitive to sound and vibrations up to the time of death. Fish which remained in anesthesia throughout a test were placed in fresh water until they recovered. The times for recovery and survival were noted.

The toxicity data were analyzed according to the methods of Litchfield and Wilcoxon (1949) to determine LC50's, variations, slope functions, and 95 percent confidence intervals.

Safety indexes were calculated to determine the margin of safety between efficacious and lethal concentrations of quinaldine. The values

Table 2.--Quantities of salts added to deionized water at the Fish Control Laboratories

Classification of water	Salt added in mg./l.				pH range	Concentration as ppm CaCO ₃	
	NaHCO ₃	CaSO ₄	MgSO ₄	KCL		Total hardness	Total Alkalinity
Soft.....	12	7.5	7.5	0.5	6.4-6.8	10-13	10-13
Standard ^{1/}	48	30.0	30.0	2.0	7.2-7.6	40-48	30-35
Medium....	192	120.0	120.0	8.0	7.6-8.0	160-180	110-120
Hard.....	384	240.0	240.0	16.0	8.0-8.4	280-320	225-245

^{1/} Standard reconstituted water used in routine bioassay.

derived are the quotients of effective and lethal concentrations.

RESULTS

SPECIES AND SIZES OF FISH

Quinaldine is toxic to coldwater and warm-water fish in 96-hour exposures, and LC50's range from 2.0 to 24.9 ppm (table 3). Channel catfish are the most resistant species irrespective of size and duration of exposure. Northern pike show a decreased resistance between LC50's of 20 ppm at 24 hours and 2 ppm at 96 hours. The decrease may be attributed to a combination of starvation and quinaldine intoxication. This species demands a large and constant supply of food, but none is supplied in the bioassay.

The small sizes of rainbow trout, brown trout, lake trout, channel catfish, and large-mouth bass are more sensitive to quinaldine than large individuals, particularly at 96-hour exposures (table 3). The LC50 for 2-inch rainbow trout, for example, is 5.0 ppm while the LC50 for 6-inch fish is 15.3 ppm; and the LC50's for 2- and 6-inch brown trout are 3.5 and 14.0 ppm, respectively.

The 2-inch rainbow trout are more resistant at 24 and 48 hours in exposures to quinaldine, than at 96 hours. Two-inch brown trout respond similarly. The LC50's for lake trout, on the other hand, do not vary significantly between the 48- and 96-hour exposures.

The toxicity of quinaldine was relatively uniform to brook trout of the sizes tested. Larger individuals appeared to be more sensitive, but also had become infected with furunculosis just prior to the bioassays. The added stress factor may explain these results. The disease in these fish was diagnosed following the tests. In general, brook trout resistance to the anesthetic was similar to that of the larger rainbow and brown trout.

EFFECTS OF TEMPERATURE

In 1- to 6-hour tests at 7°, 12°, and 17°, rainbow trout are more resistant to the toxic

effects of quinaldine at 17° than at 12° or 7° (table 4). This relationship is reversed at 24 hours, and rainbow trout are more sensitive at 17°. The 1- and 96-hour LC50's range from 17.8 to 13.3 ppm at 7° and from 23.8 to 1.9 ppm at 17°. The 96-hour LC50's show significant differences in toxicity.

Rainbow trout temperature tests were repeated several times since survival and mortality were erratic and results were difficult to analyze statistically. The 12° test in table 4, for instance, indicates 50-percent mortality at 5 ppm. Concentrations of 6 to 12 killed all test animals while one of ten survived 14, 18, and 20 ppm at 96 hours. These data indicate that quinaldine is inconsistent in its toxic effects at high concentrations.

Higher temperatures of 12°, 17°, and 22° were used to determine the effects of quinaldine on bluegills (table 5). The LC50 of 10.1 ppm for 24, 48, and 96 hours indicates that exposures over 24 hours do not increase the toxicity. The effect of exposure was also small at 17° but bluegills are more resistant than at 12° or 22°. The toxicity of quinaldine at 22° increases significantly at 96 hours exposure and bluegills die at approximately 6 ppm.

EFFECTS OF WATER QUALITY

Quinaldine toxicity to 2-inch rainbow trout is essentially the same in standard and medium quality water after 1, 3, 6, and 96 hours exposure (table 6). Quinaldine is significantly less toxic in soft water, however, in 1- to 6-hour exposures. It is also less toxic in soft water at 96 hours, but the difference in soft, standard, and medium water quality is not significant at this time interval. Toxicity increases with exposure time at every water quality.

Rainbow trout survival in soft and medium quality water was erratic and some fish lived in surprisingly high concentrations of drug. In water of medium hardness one trout survived 20 ppm of quinaldine, but none survived concentrations between 6 and 20 ppm. In soft water one trout survived 18 ppm of the anesthetic while none survived concentrations between 8 and 18 ppm. Survival occurred at the higher concentrations,

Table 3.--Toxicity of quinaldine to fish at 12° C.

<u>Species</u>	<u>Average weight (grams)</u>	<u>Approximate length (inches)</u>	<u>LC50 (ppm) and 95 percent confidence interval at</u>		
			<u>24 hours</u>	<u>48 hours</u>	<u>96 hours</u>
Rainbow trout....	1.0	2	18.7 18.2-19.2	17.8 16.4-19.3	5.0 4.5-5.6
Do.....	23.0	6	16.0 14.2-18.1	15.3 13.9-16.8	15.3 13.9-16.8
Brown trout.....	2.6	2	13.0 10.9-15.5	9.0 6.0-13.5	3.5 2.1-5.9
Do.....	14.3	4	18.0 15.3-21.2	17.0 14.9-19.4	16.0 14.7-17.4
Do.....	27.0	6	15.0 13.6-16.5	14.8 13.5-16.3	14.0 12.7-15.4
Brook trout.....	12.5	3	14.5 13.1-16.1	14.0 12.8-15.3	13.6 12.5-14.8
Do.....	20.0	4	15.0 13.2-17.1	14.0 13.1-15.0	13.5 12.6-14.4
Do.....	37.5	6	13.2 12.5-14.0	12.4 11.4-13.5	12.0 10.7-13.4
Lake trout.....	2.0	2	6.8 5.8-8.0	5.6 5.0-6.3	5.6 5.0-6.3
Do.....	5.6	3	14.2 13.4-15.1	13.5 12.4-14.7	13.5 12.4-14.7
Do.....	35.0	7	13.0 12.1-13.9	12.6 11.7-13.6	12.3 11.3-13.4
Northern pike....	1.8	2	20.0 18.8-21.2	8.0 6.3-10.2	2.0 1.1-4.6
Channel catfish..	1.9	3	21.0 19.3-22.9	20.0 18.2-22.0	19.9 18.1-21.9
Do.....	5.2	4	29.4 28.3-30.6	27.4 25.6-29.3	24.9 23.3-26.6
Bluegill.....	1.3	2	10.1 9.4-10.8	10.1 9.4-10.8	10.1 9.4-10.8
Do.....	2.8	3	12.8 12.2-13.4	12.8 12.2-13.4	12.6 11.8-13.5
Largemouth bass..	0.5	1	10.4 9.7-11.1	9.4 8.5-10.3	4.6 3.5-6.1
Do.....	5.2	4	10.4 9.8-11.0	9.9 9.3-10.5	6.5 5.6-7.5

(continued)

Table 3.--Toxicity of quinaldine to fish at 12° C.

Species	Average weight (grams)	Approximate length (inches)	LC50 (ppm) and 95 percent confidence interval at		
			24 hours	48 hours	96 hours
Largemouth bass..	63.0	7	10.0 8.8-11.3	9.7 8.7-10.9	9.0 7.7-10.5
Walleye	0.7	2	10.1 9.4-10.9	10.1 9.3-11.0	9.8 8.9-10.8

Table 4.--Toxicity of quinaldine to rainbow trout at three temperatures

Temperature ° C.	LC50 (ppm) and 95 percent confidence interval at					
	1 hour	3 hours	6 hours	24 hours	48 hours	96 hours
7°.....	17.8 16.2-19.4	16.1 15.6-16.7	16.1 15.6-16.7	15.5 14.3-16.8	14.2 12.6-15.9	13.3 11.9-14.9
12°.....	19.8 18.8-20.8	19.8 18.8-20.8	19.8 18.8-20.8	18.7 18.2-19.2	17.8 16.4-19.3	5.0 4.5-5.6
17°.....	23.8 21.5-25.4	23.8 21.5-25.4	23.0 20.3-26.0	8.0 6.2-10.1	3.2 2.3-4.5	1.9 1.5-2.3

Table 5.--Toxicity of quinaldine to bluegills at three temperatures

Temperature ° C.	LC50 (ppm) and 95 percent confidence interval at		
	24 hours	48 hours	96 hours
12°....	10.1 9.4-10.8	10.1 9.4-10.8	10.1 9.4-10.8
17°....	12.5 11.9-13.1	11.8 10.9-12.7	11.6 10.7-12.5
22°....	11.3 10.6-12.1	11.0 10.0-12.1	5.8 5.6-6.0

but mortality was not erratic or unusual at the lower end of the lethal range. The trials in various water qualities were repeated several times to confirm the variations in survival.

RECOVERY

Fish exposed to sublethal concentrations of quinaldine usually recovered from anesthesia in the test vessel within the 96-hour bioassay.

Anesthesia progresses to partial or total loss of equilibrium within 15 to 30 minutes. The effects remain for 3 to 6 hours and then diminish. Intermediate concentrations produced anesthesia much faster and killed fish at progressively higher concentrations. Fish surviving the partial kill range, but still in deep anesthesia at 96 hours, were removed to fresh water and recovery was noted. Recovery was considered complete when the fish could swim against a current.

Table 6.--Toxicity of quinaldine to rainbow trout in selected water qualities at 12° C.

Water quality ^{1/}	LC50 (ppm) and 95 percent confidence interval at					
	1 hour	3 hours	6 hours	24 hours	48 hours	96 hours
Soft.....	25.0 21.8-28.7	25.0 21.8-28.7	24.1 20.8-27.8	19.0 17.1-21.2	17.0 15.3-18.8	5.1 4.4-6.0
Standard.	19.8 18.8-20.8	19.8 18.8-20.8	19.8 18.8-20.8	18.7 18.2-19.2	17.8 16.4-19.3	5.0 4.5-5.6
Medium...	19.1 17.4-21.0	18.5 16.9-20.3	18.4 16.9-20.1	16.7 15.2-18.3	14.5 12.6-16.7	4.6 4.2-5.1
Hard.....	21.1 18.5-24.0	20.9 18.4-23.8	20.9 18.4-23.8	17.2 13.8-21.5	9.9 6.8-14.4	4.3 3.7-5.0

^{1/}Water qualities correspond to table 2.

Northern pike and channel catfish which survive partial kill concentrations appear to recover faster than the other species and without additional mortality (table 7). The partial kill concentrations for these species are relatively high. Considering all species, the recovery is approximately 95 percent after 96-hour exposures, and all except bluegills recover quite rapidly. Occasionally one or several of a group will require longer periods. This fact was noted especially among lake trout and largemouth bass.

SAFETY INDEXES

The safety indexes for rainbow trout indicate greater safety in 15-minute exposures than in 30- or 60-minute exposures (table 8). The EC50 refers to the concentration of quinaldine producing total loss of equilibrium in one-half of the specimens. This stage and other stages of anesthesia are described by Schoettger and Julin (1967).

The maximum safety index is based on concentrations producing 99-percent effective anesthesia (EC99) and 1-percent mortality (LC1). These values were extrapolated from the regressions used in determining the EC50 and LC50. The maximum safety index is lower than the safety index and is biased in favor of greater safety. Maximum safety indexes also indicate greater safety in 15-minute exposures (table 8). Indexes for 30- and 60-minute exposures are 0.9 and 1.0 and appear marginal

for practical applications of quinaldine. Values less than 1.0 indicate that one must expect mortality if 99 percent of the fish are anesthetized.

DISCUSSION

When the toxicities of quinaldine and MS-222, another fish anesthetic are compared, it is apparent that both drugs elicit similar patterns of toxic response among various species and sizes of fish. Previous research indicates that channel catfish are more resistant than other species to quinaldine and MS-222 (Marking, 1967, and Schoettger et al. 1967). Smaller sizes of fish are generally more sensitive to the anesthetics, and rainbow trout respond erratically in waters of various qualities. Among the trout, lake trout are more sensitive to both anesthetics. The drug safety indices indicate that shorter exposures are safer and minimize mortality.

Actually, quinaldine is more toxic to fish than MS-222. The LC50's for quinaldine range from 2.0 to 24.9 ppm while those for MS-222 range from 32.0 to 62.1 ppm for all species tested in 96-hour exposures at 12°. However, quinaldine is effective as an anesthetic at lower concentrations than MS-222 so that the safety indexes for both are quite similar.

In contrast with MS-222 quinaldine is more toxic at colder temperatures to 2-inch rainbow trout in 1- to 6-hour exposures. Lethal intoxication is not apparent in brief exposures at warmer temperatures, but toxic effects are

Table 7.--Recovery from anesthesia of fish exposed to concentrations of quinaldine causing partial kills within 96 hours at 12° C.

Species (all sizes)	Partial kill concentration (ppm)	Number of fish		Minutes to recover in fresh water
		Surviving at 96 hours	Recovering after 96 hours	
Rainbow trout ..	5-16	105	102	2-60
Brown trout.....	1-16	53	49	4-90
Brook trout.....	10-16	43	42	3-60
Lake trout.....	6-14	24	21	4-120
Northern pike...	10-24	24	24	5-35
Channel catfish.	12-36	97	97	4-16
Bluegill.....	9-14	73	71	19-75
Largemouth bass.	2-11	68	60	5-120
Walleye.....	3-10	38	35	5-60

Table 8.--Safety and maximum safety indexes of quinaldine against 2-inch rainbow trout in brief exposures at 12° C.

Exposure (minutes)	Safety index			Maximum safety index		
	LC50 (ppm)	EC50 (ppm)	LC50/EC50	LC1 (ppm)	EC99 (ppm)	LC1/EC99
15....	35.2	13.6	2.6	25.0	20.0	1.3
30....	25.2	14.0	1.8	18.5	20.0	0.9
60....	22.4	13.3	1.7	17.0	16.4	1.0

manifest after 24 hours. Quinaldine is also more toxic to bluegills at higher temperatures but only after 48 hours of exposure.

Quinaldine is less toxic to 2-inch rainbow trout in soft than in harder waters. Marking (1967) found little difference in the toxicity of MS-222 to rainbow trout at various water hardnesses. The low salt content and correspondingly low pH value of the soft water apparently affect the activity of quinaldine. Schoettger and Julin (1968) observed the effects of pH on quinaldine and noted that the drug is completely ineffective on fish at pH 5°. These data agree with the decreased toxicity of quinaldine in soft water.

CONCLUSIONS

Quinaldine is toxic to fish, and the 96-hour LC50's for nine species range from 2.0 to 24.9 ppm. Channel catfish are the most resistant to the drug.

The anesthetic is more toxic to small fish, especially at 96 hours exposure. It is also more toxic to small rainbow trout and bluegills at higher temperatures in the longer exposure period.

Small rainbow trout respond erratically to the toxicity of quinaldine at various temperatures and water qualities. The fish are more resistant to the drug at higher temperatures in 1- to 6-hour exposures but less resistant in 24- to 96-hour exposures. They are also more resistant to the anesthetic in soft water than in harder waters.

The safety indexes for quinaldine on fish indicate that brief exposures are safer for the fish and minimize mortalities.

Recovery from anesthesia is good among survivors exposed to partial-kill concentrations of quinaldine for 96 hours. The recoveries of nine species in fresh water occur within 2 to 120 minutes. The process of recovery from anesthesia in static test solutions begins during the bioassay after 6 hours of exposure.

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INVESTIGATIONS IN FISH CONTROL

**24. Quinaldine as an Anesthetic
for Brook Trout, Lake Trout,
and Atlantic Salmon**

By David O. Locke



UNITED STATES DEPARTMENT OF THE INTERIOR
Fish and Wildlife Service
Bureau of Sport Fisheries and Wildlife
Washington, D.C. • January 1969

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QUINALDINE AS AN ANESTHETIC FOR BROOK TROUT, LAKE TROUT, AND ATLANTIC SALMON

By David O. Locke

Maine Department of Inland Fisheries and Game, Augusta

ABSTRACT. --Quinaldine (2-methylquinoline) was an effective anesthetic for yearling Atlantic and landlocked salmon and brook and lake trout in waters ranging from 10 to 40 ppm total hardness and temperatures ranging from 36° to 40° F. and from 47° to 59° F. Lake trout were more sensitive than the other species tested. In tests, anesthetization and recovery rates for five concentrations (5, 10, 15, 20, and 25 ppm) at both temperatures (10 ppm) was generally satisfactory for lake trout. A concentration of 15 ppm was satisfactory for marking and general handling of salmon and brook trout. Quinaldine is one twenty-fourth as expensive as MS-222 at 1:12,000, and in view of our excellent results this drug warrants wider use as a fish anesthetic.

The comparatively high cost of the popular MS-222 as an anesthetic for fish has prompted many fishery workers to consider less costly drugs as substitutes. This paper reports on tests of quinaldine for anesthetizing several coldwater fishes. Quinaldine (2-methylquinoline) is currently available in practical grade from Distillation Products Industries, Division of Eastman Kodak Company, at \$14.35 per 500-gram bottle. Quinaldine is also used in the manufacture of dyes and explosives. It has not been used in medicine as have other quinolines, but according to Muench (1958) it may have some antiseptic value. The exact mode of action of quinaldine on fish is unknown, but it supposedly acts like barbiturates, depressing the central nervous system, especially the respiratory center.

Although quinaldine is used extensively by fishery workers as an anesthetic, little has been published on its use. Muench (1958) reported its use on green sunfish, white crappie, yellow bullhead, golden shiner, and goldfish. Leitritz (1962) mentioned that the concentrations used range from 5 to 12 ppm. Bell (1964) recommended doses of 6.6 to 10 ppm for 10-inch coho salmon. We decided to determine the usefulness of quinaldine for anesthetizing the commonly handled salmonids in the soft waters found in Maine.

I wish to thank Donald F. Mairs for his assistance and advice. Dr. W. Harry Everhart, Robert E. Foye, and Robert S. Rupp critically reviewed the manuscript and made many helpful suggestions.

MATERIALS AND METHODS

A stock solution was prepared by mixing 37.85 milliliter of quinaldine with 40 milliliter of acetone and enough distilled water to make 1 liter. The stock solution maintains its effectiveness for long periods when stored in brown bottles. One milliliter of stock solution added to 1 gallon of water gives a concentration of 10 ppm.

Tests were conducted on yearling Atlantic and landlocked salmon (Salmo salar), brook trout (Salvelinus fontinalis), and lake trout (S. namaycush) in waters ranging from 10 to 40 ppm total hardness and at temperatures ranging from 36° to 40° F. and from 47° to 59° F. Five concentrations were tested: 5, 10, 15, 20, and 25 ppm. Each test was performed in duplicate. Control fish were handled exactly the same as test fish except that they were placed in containers of untreated fresh water. Each test consisted of placing six fish in a Fernow pail containing 5 gallons of solution. We recorded the time required for fish to recover from anesthesia by

placing them in wash tubs containing 5 gallons of fresh water. All fish, including the controls, were fin-clipped for identification and subsequently held in raceways for 2 weeks to observe delayed mortality.

Each fish was considered anesthetized when it remained quietly on the bottom of the pail and exhibited no movement other than respiration and an occasional flexure of the caudal fin. Recovery was considered complete when the fish righted itself and maintained its equilibrium. Anethetization time was recorded for the first, third, and last fish. Each treatment was terminated after the fish had been in the test solution for 15 minutes. All fish were then placed in fresh water, and recovery time of the first, third, and last fish was recorded.

RESULTS

Anesthetization and recovery rates of different kinds and sizes of fish were directly proportional to the concentration of quinaldine in both temperature ranges (figs. 1-4). Generally the fish were anesthetized quicker at the higher temperature and also recovered sooner than at the lower temperature. The greatest contrast in rate of anesthetization relative to concentration occurs at the lower temperatures. It takes 5 to 7 times as long to anesthetize brook trout at 5 ppm than at 10 or 15 ppm at 36° F. Although the efficacy was not affected to this degree on the other species, the 5 ppm concentration did not provide dependable and speedy anesthetization for these ranges in temperature.

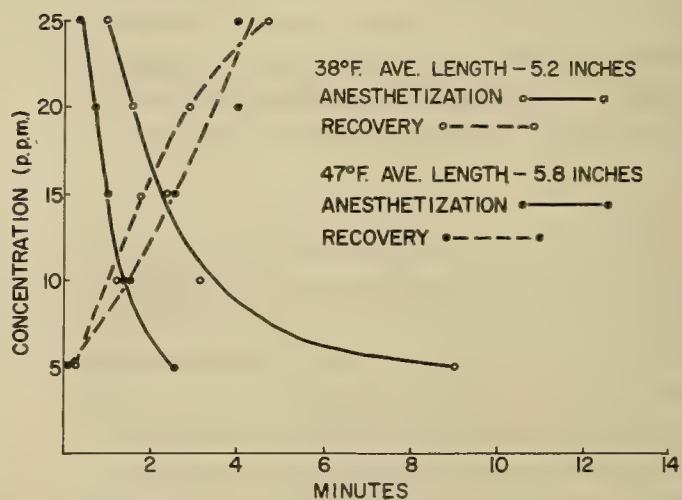
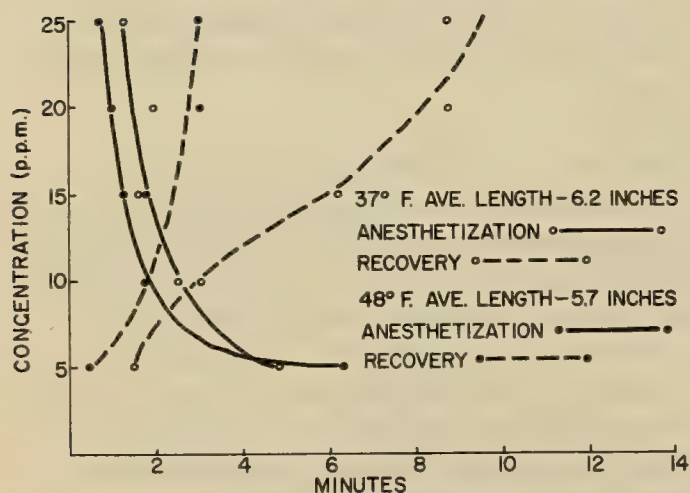
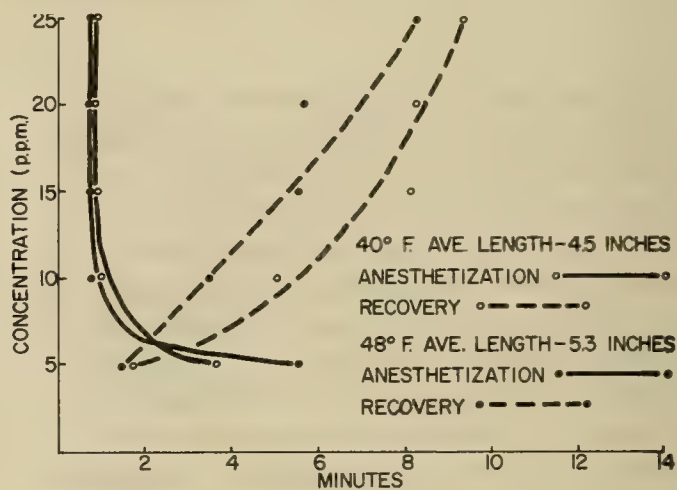
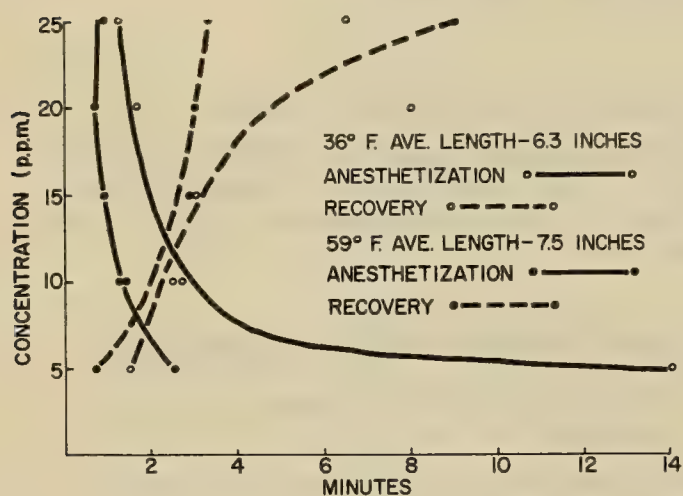


Figure 1.--Effect of quinaldine on brook trout (top left), lake trout (top right), Atlantic salmon (bottom left), and landlocked salmon (bottom right) at two temperature levels. Reported times are averages of two trials and indicate the anesthetization and recovery of one-half of the test fish.

The muscles of anesthetized fish became relaxed and the fish did not respond to gentle handling. Rough handling and sudden disturbances sometimes caused the anesthetized fish to swim a short distance and then come to rest again. Larger fish appeared to be more deeply narcotized than smaller ones.

The respiration rate of effectively anesthetized fish appeared to be more rapid than that of the control fish. Opercular movements were slight. Respiratory movements did not cease among any of the species at any of the concentrations tested.

Anesthetized fish retained their dark coloration, while the control fish became very light in response to the light background of the test container. This failure to change color is probably the result of the anesthetic upon the central nervous system.

Recovery began with a shivering movement that gradually increased in intensity until the fish had recovered fully. Lake trout gasped at the surface during recovery.

Generally, the anesthetization rates at the higher temperatures were only slightly greater than those in the lower temperatures. Recovery rates were notably higher in warmer water, presumably because of the increased rate of metabolism. The recovery rates in both temperature ranges were well within acceptable limits for all concentrations tested.

Muench (1958) reported that fish exposed to effective concentrations of quinaldine for as long as 2 and 3 days recovered within a few minutes when transferred to fresh water. He also stated that green sunfish held for 11 hours in a concentration three times greater than that necessary for anesthesia suffered no ill effects. In our experiments, all fish were marked and held for 2 weeks for observation of delayed mortality. None was observed.

CONCLUSIONS

Lake trout were more sensitive to anesthesia with quinaldine than were the other species tested. Under most conditions, a

concentration of 10 ppm is satisfactory for lake trout. Salmon and brook trout are more tolerant to quinaldine than lake trout, so a concentration of 15 ppm is suggested for these species.

Quinaldine at 15 ppm is 24 times cheaper than MS-222 at 1:12,000. In view of our excellent results and the difference in cost, this drug warrants wider use as a fish anesthetic.

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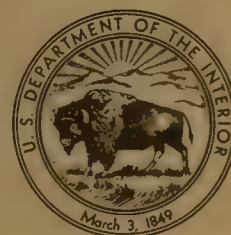
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INVESTIGATIONS IN FISH CONTROL

**25. Field Trials of Antimycin
as a Selective Toxicant
in Channel Catfish Ponds**

By Ralph M. Burress, Fishery Biologist and
Charles W. Luhning, Physical Science Technician



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FIELD TRIALS OF ANTIMYCIN AS A SELECTIVE TOXICANT IN CHANNEL CATFISH PONDS

By Ralph M. Burress, Fishery Biologist
and Charles W. Luhning, Physical Science Technician
Bureau of Sport Fisheries and Wildlife
Southeastern Fish Control Laboratory, Warm Springs, Georgia

ABSTRACT.--Antimycin effectively and economically controlled heavy infestations of green sunfish and golden shiners from selected channel catfish ponds at a Mississippi fish farm. An initial application of 5 p.p.b. of antimycin in two ponds and 7.5 p.p.b. in the third pond eliminated nearly 99 percent of the scalefishes. A followup treatment of 10 p.p.b., 4 days later, further reduced these populations with no apparent effect on yearling catfish. At harvest, three untreated ponds produced 1,474 pounds of scalefishes or an average of 389 pounds per acre, yielded 27.4 percent or 1,155 fewer catfish than the three treated ponds, and contained 1,183 undersize fish or nearly three times as many that were too small for table use. Comparison of the adjusted yields of catfish from treated and untreated ponds, ranging in size from 0.94 to 1.39 acres, indicates that treated ponds produced an additional 1,015 pounds of fish worth \$507.50, while antimycin cost only \$145.79 -- a net return of \$2.48 for each dollar invested in toxicant.

To those engaged in commercial production of channel catfish, the presence of undesirable species of scalefishes in rearing ponds is a potentially serious problem. The Bureau of Commercial Fisheries (1966) reports that about 35 million pounds of catfish are raised annually in the United States, and predicts that commercial production can reach 60 million pounds per year. The rapidly increasing demand has induced numbers of pond owners to undertake catfish production, but many have experienced losses caused by undesirable species of fish in broodponds and rearing ponds. Meyer (1965) discussed the nature of these losses, and reported the results of experiments in which various organophosphate insecticides were tested to determine whether they could be utilized for selective removal of

trash fish from ponds used to produce catfish or bigmouth buffalo.

The purpose of this study was to evaluate the effectiveness of antimycin in controlling undesirable scalefishes in soft-water ponds used for commercial production of catfish, and to measure benefits derived from the treatment. The work was done in 6 of 14 dug ponds at a private fish-farm in Columbus, Miss., during the period August 1, 1966, to January 25, 1967.

Our sincere thanks are expressed to the following whose cooperation made this study possible: Mr. Ruben Prescott, owner of the ponds; Wisconsin Alumni Research Foundation, owner of antimycin; and Ayerst Laboratories, New York, producer of antimycin.

METHODS AND MATERIALS

EXPERIMENT DESIGN

The owner of the farm assisted us in selecting three pairs of ponds similar in size, depth, severity of infestation by scalefishes, and average size of channel catfish.

The field work was done in two stages. The first stage, done in August, included (1) sampling of the fish populations in six ponds by seining, (2) applying antimycin in the Fintrol-5 formulation to three ponds, and (3) recovering scalefishes from the treated ponds. The second stage, in October and January, involved (1) draining all ponds, (2) analyzing fish populations, and (3) evaluating the results of the treatment.

DESCRIPTION OF PONDS

The ponds ranged from 0.94 to 1.39 acres in surface area (table 1). They were steep sided and each had a scooped catch basin at the deep end measuring about 15 by 40 by 2 feet. The maximum depth outside the catch basin in the first two pairs of ponds was about

TABLE 1.--Some physical and chemical characteristics of three ponds treated with antimycin

Characteristic	Pond 1	Pond 3	Pond 5
Surface area.....acres.	0.94	1.26	1.17
Average depth.....feet..	5.25	5.20	3.50
Volume.....acre-feet..	4.94	6.93	4.10
Secchi disk transparency..inches..	7	12	14
Temperature (celsius) at 7:30 a.m:			
Aug. 1.....	30°	30°	30°
2.....	30°	30°	30°
3.....	28°	28°	28°
pH Aug. 1:			
7:45-8:30 a.m.....	8.2	7.3	7.7
2:30-3:00 p.m.....	10.1	9.4	9.4
5:45-6:30 p.m.....	10.2	--	--
pH Aug. 2:			
7:45-8:30 a.m.....	8.5	7.5	7.1
2:30-3:00 p.m.....	9.9	9.4	9.4
5:45-6:30 p.m.....	9.7	--	9.4
pH Aug. 3:			
7:45-8:30 a.m.....	7.3	7.5	6.9
2:30-3:00 p.m.....	--	9.5	--
5:45-6:30 p.m.....	--	9.2	--

TABLE 2.--Characteristics of water in three treated ponds¹

Characteristic	Pond 1	Pond 3	Pond 5
Resistivity (26.6° C.).....	11,500	12,500	13,000
pH.....	10.5	10.3	10.8
Alkalinity, total (p.p.m.).....	22.0	19.0	20.0
Total hardness (p.p.m.).....	20.0	20.0	14.0
Calcium hardness (p.p.m.).....	9.2	10.0	6.0
Sulfate ion (p.p.m.).....	4.5	7.0	4.0
Total phosphorus (p.p.m.).....	0.1	0.2	0.5
Total iron (p.p.m.).....	0.9	1.4	1.0
Nitrite nitrogen (p.p.m.).....	Trace ²	0.02	0.28
Nitrate nitrogen (p.p.m.).....	0.3	0.3	1.1

¹ Samples taken at 4:00 p.m., August 8, refrigerated, and tested at Warm Springs on August 10 by standard methods of analysis.

² Trace = less than 0.01 p.p.m.

7 feet, while that in the third pair was only 5 feet.

These ponds contained relatively soft water with total hardness ranging from 14 to 20 p.p.m. (as CaCO₃), and pH values were quite high (table 2). The other chemical characteristics indicate that Pond 5 was somewhat more fertile than Ponds 1 and 3. Ponds 1 and 5 received water from a well, while Pond 3 was filled from a deep, open pit which contained scalefishes.

None of the ponds contained growths of filamentous algae or submersed aquatic vegetation. However, each supported a dense bloom of phytoplankton, which caused marked rises in pH values each day. The addition of 25 pounds of commercial catfish feed per day per pond contributed to development of the blooms, and produced noticeable accumulations of organic matter on the bottom in the feeding areas.

Each pond was stocked in 1965 or 1966 with about 2,000 fingerling channel catfish (*Ictalurus punctatus*). In addition, all the ponds contained golden shiners (*Notemigonus crysoleucas*) and green sunfish (*Lepomis cyanellus*). The open-pit water supply was populated by golden shiners, warmouth bass (*Chaenobryttus gulosus*), green sunfish, and bluegills (*Lepomis macrochirus*).

SAMPLING AND HARVESTING

Average lengths and weights of the catfish at the time of treatment were calculated from a sample of about 50 fish from each pond

TABLE 3.--Numbers, weights, and percentages of channel catfish sampled in August in each of three pairs of ponds; the designations (T) and (U) identify treated and untreated ponds

Pond	Date of stocking	Number of fish	Percent of population sampled ¹	Weights (lbs.)	
				Total	Average
1 (T)....	10/23/65	48	2.4	33.40	0.70
2 (U)....	9/06/65	51	3.1	44.00	0.86
3 (T)....	10/23/65	47	2.8	24.25	0.52
4 (U)....	2/09/66	29	3.0	6.25	0.22
5 (T)....	11/09/65	56	3.4	17.50	0.31
6 (U)....	11/09/65	47	2.9	19.25	0.41

¹ Based on number of fish recovered at harvest.

(table 3). When possible, the fish were taken with a 30-foot, 1-inch-mesh seine, which was stretched out just beyond the feeding area and hauled in rapidly while the fish were feeding. This technique worked very well in Ponds 5 and 6, which are comparatively shallow, but in the deeper ponds these samples had to be augmented by catches made with a 125-foot, 1-inch-mesh seine or with a 250-foot, 1-inch-mesh gill net. The size of the sample in Pond 4 was reduced because the water was too deep for effective seining, and gill netting would have killed too many fish. The reduction in sample size was not regarded as serious, since the fish were uniform in length and weight.

Scalefish populations were sampled to obtain an estimate of their abundance in each pond. Two 40-foot hauls were made with a 20-foot, 1/4-inch-mesh seine, and the fish were enumerated by species and discarded (table 4).

Five of the six ponds were harvested in January 1967. All of the catfish were counted and weighed. We also made a fairly accurate measure of the scalefish even though some were lost in the mud.

Pond 2 was drained by the owner of the fish farm on October 3 to meet an urgent need for catfish in his restaurant. The number and dressed weight of the catfish were recorded, and the live weight was computed from these figures. Although scalefishes were not collected at the time of harvest, an estimate of their numbers was made.

TABLE 4.--Number of scalefish of each size group sampled by seining in each of three pairs of ponds (the two size groups represent fish hatched in pond and initial invaders)

Pond	Green sunfish		Golden shiners		Tadpoles
	1-1.5 in.	2-6 in.	1.5-2.25 in.	3-4.5 in.	
1....	321	6	1,510	1	0
2....	338	14	543	0	0
3....	1,410	0	0	0	238
4....	547	50	16	0	0
5....	653	13	25	0	0
6....	scores ¹		hundreds ¹		

¹ Seining data misplaced.

APPLICATION OF TOXICANT

At the time of treatment, the water temperature was above 60° F. and the pH was less than 8.5, hence a concentration of 5 parts per billion (p.p.b.) of antimycin in the Fintrol-5 formulation was selected in accordance with the manufacturer's recommendation. The toxicant was applied from a boat with a hand-operated seed spreader. Treatment of each pond was accomplished in 15-20 minutes between 7:45 and 9:00 a.m. on August 2. We added a supplementary treatment of 2.5 p.p.b. of antimycin to Pond 1 at 9:30 a.m. when we discovered that the pH had risen to 8.5 by 8:30 a.m.

We observed on August 4 that a few scalefish had survived in treated ponds, hence 10 p.p.b. of antimycin were applied to each pond on August 5 in an effort to determine the remnant populations and obtain a better evaluation of the results of the first treatment. These applications were completed between 6:05 and 7:15 a.m., which gave the antimycin almost 2 hours longer to take effect before the diurnal elevation in pH occurred. We recovered all the dead fish within 3 days after each treatment.

DEGRADATION OF TOXICANT

Golden shiners and fingerling green sunfish were seined from Pond 6, and 10 fish of each species were placed in live-boxes in the treated ponds about 24 hours after each application of the toxicant. We considered that degradation of the toxicant was complete when all of the fish survived a 48-hour exposure in the treated water.

RESULTS

FIRST TREATMENT

Small green sunfish and golden shiners were surfacing and gulping air within 15 minutes after we completed the applications of antimycin. Within 3 hours adult fish of both species began surfacing, and some could be taken easily with a dip net. Most of the golden shiners and a substantial proportion of the small green sunfish were recovered on the day of treatment. Larger fish of each species were slower to appear.

We observed several green sunfish in each pond which were too alert and active to be captured by dip net at 6:00 p.m. on the day of treatment. This was the first indication that the treatment was not completely successful. Although collections of fish were good for the next 2 days, the degradation of the toxicant was essentially complete within 24 hours after application as shown by tests with caged fish.

The first application of antimycin was highly effective in removing scalefish populations regardless of their composition (table 5). In Pond 1 we recovered about 187 pounds of scalefish per acre. Golden shiners were dominant, comprising 88.4 percent by number and 78.8 percent by weight. In Pond 3 fingerling green sunfish amounting to 146 pounds per acre constituted more than 99 percent of the scalefish population by number and weight. In Pond 5 the population was comparatively small, amounting to only 44 pounds per acre. Green sunfish of both sizes comprised more than 99 percent of the population by number and weight.

In order to check on the completeness of the kill, we again used the technique of seining at the time of feeding. It was interesting to see that several adult green sunfish which survived exposure to 5.0 and 7.5 p.p.b. concentrations of antimycin just 2 days earlier were not off feed, but responded with characteristic quickness to the familiar sound of food pellets hitting the water.

TABLE 5.--Total numbers and weights in pounds (in parentheses) of scalefish recovered following the first and second applications of antimycin

Species and size groups	Pond 1			Pond 3			Pond 5		
	First (7.5 p.p.b.)	Second (10 p.p.b.)	Total	First (5 p.p.b.)	Second (10 p.p.b.)	Total	First (5 p.p.b.)	Second (10 p.p.b.)	Total
Golden shiner: 1.5-4.5 inches.....	31,435 (138.2)	2 (Trace) ¹	31,437 (138.2)	25 (0.3)	12 (0.1)	37 (0.4)	65 (0.3)	0	65 (0.3)
Warmouth: 4-7 inches.....	3 (0.4)	7 (1.5)	10 (1.9)	0	0	0	0	0	0
Green sunfish: 1-1.5 inches.....	3,858 (20.2)	9 (0.1)	3,867 (20.3)	59,840 (178.0)	1,019 (5.5)	60,859 (183.5)	9,526 (19.8)	3 (Trace)	9,529 (19.8)
2-6 inches.....	192 (10.4)	52 (4.2)	244 (14.6)	2 (0.3)	3 (0.3)	5 (0.6)	1,133 (31.3)	15 (0.8)	1,148 (32.1)
Bluegill: 8 inches.....	1 (0.5)	0	1 (0.5)	0	0	0	0	0	0
TOTAL.....	35,489 (169.7)	70 (5.8)	35,559 (175.5)	59,867 (178.6)	1,034 (5.9)	60,901 (184.5)	10,724 (51.4)	18 (0.8)	10,742 (52.2)

¹ Trace = less than 0.1 lb.

SECOND TREATMENT

Within 3 hours after the treatment with 10 p.p.b. of antimycin, it was evident that the first treatment had eliminated most of the golden shiners in each pond. Our final count showed that the following percentages of golden shiners had survived: Pond 1, less than 0.01 percent; Pond 3, 32.4 percent; Pond 5, 0.0 percent. More than 99.7 percent of the small green sunfish were eliminated in Ponds 1 and 5; only 9 and 3 fish were recovered in each, respectively. In Pond 3, 1,019 small green sunfish had survived or 1.67 percent of the total number recovered.

Recovery of 52 green sunfish from 2 to 6 inches long in Pond 1 was unexpectedly high, amounting to 21.3 percent of the total number of this size group taken. In Pond 3 we found that 3 of 5 adult green sunfish had survived, while in Pond 5 we recovered only 15 of the 1,148 adult fish originally present.

Seven of 10 adult warmouth taken from Pond 1 had survived the first treatment, whereas the single large bluegill present did not.

Tadpoles began to die in Pond 3 shortly after the second treatment was made, and in 2 days we recovered about 1,990 tadpoles weighing 33.25 pounds. What percentage of the population this represented is not known, but a complete kill did not occur.

Degradation of antimycin was complete in all three ponds within 48 hours after application.

SCALEFISH IN TREATED PONDS AT HARVEST

Antimycin treatments were highly successful in that no golden shiners or warmouth were found in Ponds 1 and 3, and the reduction of green sunfish in each pond was more than 99.99 percent complete (table 6). Since there was no exchange of water between these two ponds and untreated ponds, we assume that the green sunfish had survived the treatment.

Unfortunately the exact results of the treatment of Pond 5 were obscured because an undetermined number of scalefish were carried in subsequently by an overflow from an adjacent pond. Since no golden shiners were recovered in this pond following the second application of antimycin, and since no warmouth were found following either treatment, we are virtually certain that the few individuals of each species which were observed at harvest were post-treatment invaders. The bottom of this pond was exceptionally flat, and contained scores of shallow depressions in the deep, soft mud. Thousands of green sunfish, many of which had hatched following the treatments, were trapped, and we could not make an accurate assessment of

TABLE 6.--Total numbers and total weights in pounds (in parentheses) of scalefish recovered at draining

Species and size groups	Treated ponds			Untreated ponds		
	1	3	5	2	4	6
Golden shiner: 2.5-5.5 inches.....	0	0	Very few ¹	² 50,000 (253)	1,428 (50)	31,680 (308)
Green sunfish: 1.0-3.5 inches.....	0	2 (0.2)	³ 1,000's	² 40,000 (125)	39,740 (344)	18,627 (186)
3.5-6.0 inches.....	4 (1.5)	7 (1.2)	³ 150-200	0	2,421 (178)	170 (29)
Bluegill.....	0	1 (0.5)	0	0	0	0
Warmouth.....	0	0	Very few ¹	0	0	3 (1.0)
TOTAL	4 (1.5)	10 (1.9)	--	90,000 (378)	43,589 (572)	50,480 (524)

¹ Probably carried in by overflow from adjacent pond.

² Number estimated by pond owner at draining; all sizes included.

³ Numbers estimated because many small fish were lost in soft mud; some probably carried in by overflow from adjacent pond.

the numbers of green sunfish present. An estimated 150-200 green sunfish from 3.5 to 6.0 inches in length were seen, many of which must have survived the antimycin treatments. Even if we assume that there were 200 and that none had immigrated into the pond, there was a reduction of more than 98.12 percent in the numbers of green sunfish originally present.

SCALEFISH IN UNTREATED PONDS AT HARVEST

Large numbers of scalefish were found when the untreated ponds were drained (table 6). In Pond 4 green sunfish weighing 418 pounds per acre comprised 91.3 percent of the scalefish population by weight, and large golden shiners amounting to about 40 pounds per acre made up the remainder. In Pond 6 golden shiners weighing 268 pounds per acre made up 58.8 percent of the total weight, and green sunfish weighing 187 pounds per acre comprised 41.2 percent of the total weight. The pond owner estimated that there were 40,000 green sunfish and 50,000 golden shiners of several sizes in Pond 2 at harvest. If weight data obtained during our sampling of the population 2 months prior to harvest were applied to this estimate, we can calculate that about 90 pounds of green sunfish and 182 pounds of golden shiners were removed per acre. In all three of these ponds there

were hundreds of scalefish large enough to compete directly with the catfish for food.

HARVEST OF CHANNEL CATFISH

Three treated ponds having a combined area of 3.37 acres produced 5,363 catfish weighing 4,855 pounds, and the three untreated ponds having a combined area of 3.79 acres produced 4,208 catfish weighing 3,088 pounds (table 7). The average weight of catfish in the treated and untreated ponds at harvest was 0.91 and 0.73 pounds, respectively. We sorted out the smallest fish from each pond, which weighed from about one-third to one-half pound, and placed them in a single pond for additional feeding. Among the treated ponds, the number of undersize fish ranged from 81 to 226 with a total number of 421, while in the two untreated ponds for which data are available, the numbers of such fish were 235 and 948 for a total of 1,183. The differences are quite pronounced, but not all of them can be attributed solely to the treatment. Some were caused by variations in time of stocking, degree of competition from scalefish populations, and rates of catfish survival which may have been influenced strongly by predation. In the following section, an attempt is made to evaluate the effects produced by these factors and to arrive at a conservative measure of the benefits derived from treatment.

TABLE 7.--Numbers and weights in pounds of channel catfish harvested from treated and untreated ponds

Pond	Area (acres)	Date of stocking ¹	Date of harvest	Harvest per pond				Average weight		Percent gain in weight	Undersize fish		
				Total		Per acre		August sample	At harvest		Number	Av. wt.	Percent
				Number	Weight	Number	Weight						
Treated Ponds:													
1.....	0.94	10/23/65	1/12/67	2,036	2,438	2,166	2,594	0.70	1.20	71.1	226	0.35	11.1
3.....	1.26	10/23/65	1/04/67	1,701	1,217	1,350	966	0.52	0.72	38.5	81	0.55	4.8
5.....	1.17	11/09/65	1/16/67	1,626	1,200	1,389	1,026	0.31	0.74	138.7	114	0.37	7.0
TOTAL	3.37			5,363	4,855	--	--	--	--	--	421	--	--
Untreated Ponds:													
2.....	1.39	9/06/65	10/03/66	1,625	² 1,527	1,169	² 1,099	0.86	0.94	³ 9.3		No data	
4.....	1.25	2/09/66	1/24/67	978	335	782	268	0.22	0.34	54.4	948	0.33	96.9
6.....	1.15	11/09/65	1/25/67	1,605	1,226	1,396	1,066	0.41	0.76	85.4	235	0.46	14.6
TOTAL	3.79			4,208	3,088	--	--	--	--	--	1,183	--	--

¹ Fingerling channel catfish 2.5 inches long stocked in all ponds.

² When total weight and weight per acre of catfish harvested from Pond 2 were adjusted to compensate for the difference in time of harvest, they became 1,735 and 1,248 pounds, respectively.

³ Percent gain in weight from sampling on August 1 to harvest on October 3.

DISCUSSION

This field trial demonstrated quite clearly that antimycin can be used to control scalefishes in soft-water ponds without killing catfish, and that the investment in the treatment was well compensated by greater production in treated ponds. The initial treatment was nearly 99 percent effective, and there is good reason to believe that its efficacy would have been enhanced had the application of Fintrol-5 been made at daybreak. This would have allowed a substantially greater amount of exposure time before the rapid rise in pH began to cause degradation of the toxicant. Berger, Lennon, and Hogan (1969) found that the effectiveness of antimycin against fish is influenced substantially by the pH of the medium. Results of their bioassays indicated that the concentration of antimycin required to produce a complete kill of fingerling goldfish in 96 hours at 12° C. was 0.20 p.p.b. at pH 5, 1.10 p.p.b. at pH 8, and 60 p.p.b. at pH 10.

Scalefish populations can be controlled at any time of the year, since water temperature has comparatively little effect on the efficacy of antimycin, and even fingerling catfish are not killed by recommended levels of treatment. Obviously such treatments yield greater benefits if accomplished a few days before ponds are stocked or soon thereafter.

It is thought that more green sunfish survived in Pond 5 because the water was comparatively shallow, and the bottom was completely covered by very soft mud. The sand formulation of antimycin used is designed to release the toxicant at a uniform rate as it sinks through the first 5 feet of water. Since the average depth of the pond was only 3.5 feet, a substantial percentage of the antimycin was not released before the sand sank into the mud. Therefore, we do not recommend lowering hatchery-type ponds prior to treatment if their average depths are not in excess of 5 feet.

The calculations of benefits cannot be based simply on the difference in total weights of catfish harvested from each group of ponds for two reasons: (1) Pond 6 was stocked much

later than any of the others, and survival of the fish was by far the poorest in this pond; and (2) Pond 2 was harvested far earlier than the others. Thus, the most conservative approach to assessing the benefits derived from the treatment appears to be that of direct comparison of weight gains made by catfish in each pair of ponds after the time of treatment.

In comparing catfish populations and production in the first pair of ponds, we find that untreated Pond 2 was stocked on September 6, 1965, or about 47 days before Pond 1. Thus, it is not surprising that the average weight of catfish in the untreated pond was 0.16 pounds or 22.9 percent greater at the August sampling. The advantage of earlier stocking, however, was largely negated by the tremendous increase in competition from hundreds of scalefishes which grew large enough to consume food pellets.

Although treated Pond 1 was harvested 101 days later than Pond 2, the following adjustment can be made to permit a meaningful comparison of the gains in weight after the time of treatment. The average weight of catfish in Pond 2 increased by 9.3 percent between sampling time on August 1 and harvest time on October 3, hence the weight gain of the entire catfish population during this 63-day period was about 130 pounds. By subtracting this figure from the harvest figure, we can calculate that their total weight on August 1 was about 1,397 pounds. If we then make the generous assumption that the catfish in Pond 2 would have continued to gain weight at the same rate during the increasingly cold months of October, November, and December, their total gain from August 1 to January 12 would have been 338 pounds, and their adjusted total weight in January would have been 1,735 pounds. By comparison the total weight of catfish in Pond 1 on August 1 was about 1,425 pounds, and their weight at harvest was 2,438 pounds. Thus, their gain amounted to 1,013 pounds, or 675 pounds more than the adjusted weight gain by the catfish in Pond 2.

The comparison of catfish production in the second pair of ponds is complicated by the

fact that Pond 3 was stocked on October 23, 1965, and untreated Pond 4 was stocked 109 days later on February 9, 1966. Catfish in the latter pond were further handicapped, because it contained by far the greatest population of adult green sunfish throughout the year as indicated both by seining in August and by the recovery of scalefishes at harvest. Under these circumstances it is not surprising that less than half of the original stock of catfish survived, that their average weight in August was only 0.22 pounds, or that 96.9 percent of them failed to reach marketable size by harvest time. Catfish in treated Pond 3 gained a total of 332 pounds following treatment while those in Pond 4 were able to gain a total of only 120 pounds. Thus, the fish in the treated pond gained 212 pounds more, though the same amounts of feed were offered and there were 723 fewer catfish in the untreated pond. The catfish in Pond 3 gained only 38.5 percent in weight following treatment as compared to 71.4 percent in Pond 1 and 138.7 percent in Pond 5. There was no apparent reason for their comparatively slow growth.

Ponds 5 and 6 were nearly identical in area, were stocked on the same day, were harvested only 9 days apart, and had almost identical rates of catfish survival. Hence, analysis of their production may afford the most clear-cut comparison of the benefits derived from treatment. For some unknown reason, the growth of catfish in Pond 5 prior to treatment was much poorer than that in Pond 6. At the time of August sampling, fish in the untreated pond were 32.3 percent heavier, the average weights of fish in the two ponds being 0.31 and 0.41 pounds, respectively. Following elimination of the scalefish in Pond 5, the fish grew at a much faster rate, and their average weight at harvest was 0.74 pounds as compared to an average of 0.76 pounds in Pond 6. Thus, the percentage difference in average weight was reduced from 32.3 to 2.7 percent. Furthermore, only 114 fish in the treated pond were too small for table use, while 235 of those in the untreated pond were undersize. Catfish in Pond 5 gained 696 pounds following treatment, while those in Pond 6 gained 568 pounds, for a difference of 128 pounds.

At the time of harvest, the local price for pond-reared catfish in the round was \$0.50 per pound, hence calculations of the value of the total weight of fish harvested whether of usable or unusable size are based on this figure. Antimycin costs are those of the first treatment only, because it reduced scalefish populations by nearly 99 percent even under the adverse water quality conditions which prevailed when the tests were made.

The greater amount of weight gained by catfish in treated ponds following treatment amounted to a total of 1,015 pounds of fish worth \$507.50 (table 8). The cost of antimycin used in the first treatment was \$145.79, leaving a net profit of \$361.71. Thus, for each dollar invested in antimycin there was a minimum net return of \$2.48.

Another rather easily estimated, but comparatively minor, benefit derived from treatment is the saving in the cost of food required to feed out undersize fish from both groups of ponds. Pond 3 yielded the smallest usable size fish, which had an average weight of 0.72 pounds. The 421 undersize fish from the three treated ponds were underweight by 138 pounds, while the 1,183 small fish from the two untreated ponds were underweight by 431 pounds. Thus, if we assume that 1.8 pounds of food are required to produce each additional pound of catfish, and that food cost \$125.00 per ton, it would cost \$15.52 to feed out fish from the former group of ponds, and \$48.47 to feed out those from the latter group, a difference of \$32.95. The costs of labor, transportation, pumping of water and other factors attendant upon the greater length of time required to feed out the smaller fish

TABLE 8.--Increase in total yield and market value of channel catfish resulting from use of antimycin to reduce scalefish populations in three ponds

Pond	Differential increase in yield (lbs.) ¹	Value of increased yield	Cost of antimycin	Net profit
1.....	675	\$337.50	\$58.49	\$279.01
3.....	212	106.00	54.71	51.29
5.....	128	64.00	32.59	31.41
TOTAL	1,015	\$507.50	\$145.79	\$361.71

¹ Derived by subtracting adjusted total weight gains in untreated ponds following date of treatment from those in treated ponds.

from the untreated ponds would be difficult to compute, but they undoubtedly would be much greater than the cost of food.

Another interesting approach to evaluation of the economic loss caused by the presence of competing scalefishes is possible if we arbitrarily assume that the amount of food required to produce one pound of scalefish might have produced a pound of catfish instead. On this basis, the untreated ponds could have produced an additional 1,474 pounds of catfish.

We recovered 1,155 fewer catfish from the untreated ponds, a significant factor which alone could spell the difference between operating the ponds at a profit or at a loss. Under the circumstances, we could not determine to what extent survival was reduced by scalefish predation on fingerling catfish, the vastly greater amount of competition of scalefish with catfish in untreated ponds, or factors of disease and accident which unaccountably operated to a much greater extent in the untreated ponds. Whatever the cause, the results were similar to those observed by Swingle (1959), who found that the presence of wild fishes in his experimental ponds reduced channel catfish production.

CONCLUSIONS

1. Nearly 99 percent of the scalefishes in three ponds used for channel catfish production were eliminated by an application of 5.0 p.p.b. of antimycin in two of the ponds and 7.5 p.p.b. in the third pond.
2. A followup treatment with 10 p.p.b. of antimycin further reduced the scalefish populations in all ponds and eliminated 26.4 pounds per acre of tadpoles in Pond 3.
3. The channel catfish were not harmed by the toxicant.
4. Two factors which prevented total eradication of the scalefish were: (a) pH of the water rose to high levels each afternoon

and caused rapid detoxification of the antimycin, and (b) average depth of the water in Pond 5 was only 3.5 feet, and full release of the toxicant was not achieved before the sand upon which it was formulated sank into the soft, muddy bottom.

5. The 5.0 and 7.5 p.p.b. concentrations of antimycin degraded to a level harmless to golden shiners and small green sunfish within 24 hours after application, and the 10 p.p.b. concentration degraded within 48 hours.
6. The untreated ponds, which produced an estimated 184,000 scalefish weighing nearly 1,474 pounds, yielded 1,155 fewer catfish than did the treated ponds.
7. If the second pair of ponds (Ponds 3 and 4) in which stocking was done at widely different times is excluded from consideration, the average yield per acre of channel catfish from the treated ponds was greater than the adjusted yield per acre from untreated ponds by about 464 fish weighing 558 pounds.
8. The increase in yield of channel catfish resulting from treatment amounted to 1,015 pounds of fish worth \$507.50, while the cost of Fintrol-5 for initial treatment was only \$145.79 -- a net return of \$2.48 for each dollar invested in toxicant.
9. Selective removal of scalefishes from catfish ponds can be accomplished safely and economically through the use of antimycin.

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26. Laboratory Studies on Antimycin A as a Fish Toxicant

By Bernard L. Berger, Chemist,
Robert E. Lennon, Fishery Biologist, and
James W. Hogan, Chemist



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LABORATORY STUDIES ON ANTIMYCIN A AS A FISH TOXICANT

By Bernard L. Berger, Chemist, Robert E. Lennon, Fishery Biologist,
and James W. Hogan, Chemist
Bureau of Sport Fisheries and Wildlife
Fish Control Laboratories
La Crosse, Wisconsin and Warm Springs, Georgia

ABSTRACT.--Liquid and sand formulations of antimycin A were tested in laboratory waters of various temperature, hardness, pH, and turbidity against 31 species of fresh-water fish of various sizes and life stages. Each formulation of toxicant was lethal under all water conditions to fish eggs, fry, fingerlings, and adult fish. Trouts are the most sensitive and catfishes the least sensitive. Of the 31 species, 24 succumb to 5 p.p.b. or less of the toxicant; only certain catfishes survive 25 p.p.b. The order of toxicity to various species of fish suggests that antimycin has possibilities for selective or partial control of certain unwanted fish. Although toxic to fish under ice, antimycin is more active in warm water than in cold. It is slightly more active in soft water than in hard; it is more active and persists far longer in water at pH 5 to 8 than at pH 9 or 10. It is active on fish in either clear and turbid waters, and it can be detoxified by potassium permanganate. The results contributed to registration of antimycin A in Fintrol-5 formulation as a fish toxicant.

Antimycin A in a formulation called Fintrol-5 was approved and registered as a fish toxicant in the United States and Canada in 1966. Its early promise as a fishery tool was mentioned by Derse and Strong (1963) and Loeb (1964). It was patented as a piscicide by Strong and Derse in 1964. Concurrently Walker, Lennon, and Berger (1964) made preliminary observations on its toxicity to fish and other aquatic organisms.

The subsequent intensive testing against fish in the laboratory which contributed to approval of antimycin as a fishery tool is discussed in this report. The studies included 31 fishes of various life stages in waters of different qualities and temperatures, indoor and outdoor bioassays, and liquid and sand formulations of the toxicant. The objectives were to define lethal concentrations

for fish under a variety of conditions, to assess factors which contribute to degradation of the toxicant, and to find a substance which might be used to detoxify it.

Generous cooperation was afforded throughout the investigation by Wisconsin Alumni Research Foundation, owner of patents on antimycin; Ayerst Laboratories, New York, producer of antimycin; national fish hatcheries; and the Iowa, Minnesota, and Wisconsin Conservation Departments.

METHODS AND MATERIALS

ANTIMYCIN

The toxicant used in these investigations was supplied by the Wisconsin Alumni Research Foundation and by Ayerst Laboratories as

crystals of antimycin A, 96 to 98 percent pure, or in formulations of antimycin A coated on sand. Stock solutions were prepared by dissolving 10 mg. of crystals in 100 ml. of acetone or ethanol. Although the solutions remain stable for several months if kept in cool, dark storage, fresh stocks were made biweekly.

The sand formulation Fintrol-5 contains 1 percent of antimycin A, 24 percent of Carbowax (polyethylene glycol 6000), and 75 percent of 40-mesh sand by weight. It is designed to release the toxicant into the water within a depth range of 0 to 5 feet as the particles sink to the bottom. Fintrol-15 is an experimental formulation, and the proportions of antimycin, Carbowax, and sand have not been released to us. It is supposed to release the toxicant within the first 15 feet of depth. Other formulations, such as a Fintrol-30 for greater depths, are under consideration.

Stability.--The effect of heat on the stability of antimycin was investigated. Quantities of crystalline and sand-formulated toxicant were subjected to dry heat at 200° C. in a forced-air oven for 15, 30, 60, and 120 minutes. Samples of the heated materials were then tested against fingerling rainbow trout in 96-hour bioassays. Results were tabulated at 0.25, 0.5, 1, 2, 3, 24, and 96 hours.

Detoxification.--Potassium permanganate (KMnO_4) was selected for preliminary testing as a detoxifier for antimycin in solution. Five to 500 p.p.b. of KMnO_4 were added to solutions which contained 5 p.p.b. of antimycin. Fingerling bluegills were placed in the solutions 6 or 24 hours later and their responses were noted at 6, 12, 24, 48, 72, and 96 hours to detect the extent and rate of detoxification.

BIOASSAY PROCEDURES

Static bioassays of antimycin were conducted as described by Lennon and Walker (1964) and Walker, Lennon, and Berger (1964). Some special tests required innovations.

Column tests.--A plexiglass column 8 feet high and 1 foot in diameter, with walls one-fourth-inch thick, was set up to determine

the uniformity and strength of antimycin released by the sand formulation at selected depths. The column was filled with reconstituted water at 12° C., and quantities of Fintrol were applied at the surface and allowed to sink to the bottom. Water from various depths was sampled by siphon and analyzed for the toxicant by 96-hour bioassays with rainbow trout and goldfish.

Simulated field tests.--The availability of greater quantities of antimycin in the spring of 1966 enabled us to initiate trials against communities of small and large fish in 0.01-acre concrete pools. Each pool contained 43,000 liters of pond water at a 3.5-foot depth or 24,668 liters at a 2-foot depth. Selected wild and hatchery-reared fish were stocked in pools 3 to 7 days before treatment with antimycin. Stocking densities ranged from less than 100 to 2,000 pounds per acre. Dead fish were removed daily, and the ponds were drained after each experiment to obtain full tallies of fish.

A series of warm-weather tests was made using 12 species of fish at densities from 1,000 to 2,000 pounds per acre. The concentrations of antimycin applied were 1, 2, 3, 4, 5, 10, and 20 p.p.b. at 11° to 21° C.

Juvenile and adult fish of four species were used in the cold-weather tests in the pools. Tests were made using both clear and turbid water, at 3.4° to 5.5° C.

WATER

Most bioassays were run in reconstituted deionized water (Lennon and Walker, 1964) but well water or pond water was used in some of the outdoor tests.

Temperature.--Heated or chilled water baths were used as needed to control the temperatures of indoor bioassays at 2° to 27° C. Bioassays in outdoor pools were run at ambient temperatures; some under 6 inches of ice and others at temperatures up to 30° C.

Hardness.--Total hardness of bioassay waters ranged from 20 to 400 p.p.m. Most tests took place in our standard, reconstituted

water, which has a total hardness of 40 p.p.m.

pH.--The range of pH in bioassay media was 5 to 10. The desired levels were attained by adding buffers to reconstituted water (table 1). Because the contrived levels tended to regress toward neutral, they were restored each 24 hours by adding small amounts of the buffer reagents. Close monitoring of control vessels which contained fish but no toxicant indicated the extent of change and the amount of readjustment necessary.

TABLE 1.--Buffer reagents added to reconstituted water to yield 15 liters of bioassay media of various pH levels

pH	Volume of buffer reagent ¹ (ml.)			
	1.0N NaOH	0.1M KHC ₈ H ₄ O ₄	1.0M KH ₂ PO ₄	1.0M H ₃ BO ₃
5 ±0.1....	10	510	--	--
6 ±0.1....	2	--	90	--
7 ±0.1....	10	--	30	--
8 ±0.1....	25	--	30	--
9 ±0.1....	8	--	--	30
10 ±0.1....	20	--	--	21

¹ NaOH = Sodium hydroxide.
KHC₈H₄O₄ = Potassium acid phthalate.
KH₂PO₄ = Potassium phosphate (monobasic).
H₃BO₃ = Boric acid.

Turbidity.--The activity of antimycin in the presence of suspended clay was checked in 96-hour bioassays with rainbow trout. Weighed portions of the clay were stirred into the bioassay media to produce turbidities of 1,000 and 5,000 p.p.m. (Secchi disk readings of 10 and 4 inches). The concentrations of antimycin ranged from 0.04 to 0.30 p.p.b. and the temperature was 12° C. All vessels were stirred for 30 seconds during each hour of the first 6 hours of the bioassay and at each 24 hours thereafter to keep the clay in suspension.

The performance of Fintrol-5 was tested against brown trout, goldfish, carp, bluegill, and largemouth bass in turbid water in outdoor, concrete pools at 3.4° to 5.5° C. Each pool contained about 25,000 liters of water

at a depth of about 2 feet. Four pools had 2 inches of clay-loam on the bottom and a Secchi disk reading of 6 inches. Four pools had no soil on the bottom, and the water remained clear. Three turbid pools were treated with 5.0, 7.5, and 10.0 p.p.b. of antimycin respectively; the fourth served as a control. Similar concentrations of antimycin were applied in three clear pools, and the fourth pool served as a control. Observations on mortalities of fish were made daily up to 18 days at which time we drained the pools and made a final assessment.

FISH

Most of the fish were obtained from National and State fish hatcheries, but wild specimens were used in certain tests (table 2). All were held under conditions of quarantine and pretest evaluation as described by Lennon and Walker (1964).

Eggs.--We studied the effects of antimycin on fertilized eggs of rainbow trout, northern pike, goldfish, carp, white sucker, and channel catfish. At first, groups of eggs of certain ages, green and eyed, were counted and placed in petri dishes. The dishes were then immersed in solutions of antimycin for certain lengths of time. Later, aluminum wire or saran mesh baskets, approximately 2 by 2 by 2.5 inches were used. Containers of this size were capable of holding 100 rainbow trout eggs.

The baskets of eggs were either exposed for specific periods or remained in the solutions of antimycin until the eggs perished or hatched. Those removed after short exposures were rinsed and placed in hatching jars, troughs, or Heath egg incubators. Groups of control eggs were exposed to solutions of the solvent in water and otherwise handled similarly.

Fry.--Bowfin 21 to 56 days old and rainbow trout 3 to 60 days old were exposed to antimycin in 1- or 5-gallon bioassay vessels. Channel catfish less than 1 day old and largemouth bass 1 to 2 days old were placed in petri dishes containing various concentrations of toxicant. Following exposure, the fish were removed, rinsed, and placed in fresh water for observation.

TABLE 2.--List of 31 fishes exposed to antimycin

Species	Life stage				Source	
	Egg	Fry	Fingerling	Adult ¹	Hatchery	Wild
Shortnose gar, <i>Lepisosteus platostomus</i>	--	--	X	X	X	X
Bowfin, <i>Amia calva</i>	--	X	X	X	X	X
Rainbow trout, <i>Salmo gairdneri</i>	X	X	X	X	X	--
Brown trout, <i>Salmo trutta</i>	--	--	X	--	X	--
Brook trout, <i>Salvelinus fontinalis</i>	--	--	X	--	X	--
Lake trout, <i>Salvelinus namaycush</i>	--	--	X	--	X	--
Northern pike, <i>Esox lucius</i>	X	--	X	X	X	X
Goldfish, <i>Carassius auratus</i>	X	X	X	X	X	--
Northern redbelly dace, <i>Chrosomus eos</i>	--	--	--	X	--	X
Carp, <i>Cyprinus carpio</i>	X	--	X	X	X	X
Fathead minnow, <i>Pimephales promelas</i>	--	--	--	X	X	--
Quillback, <i>Carpoides cyprinus</i>	--	--	--	X	X	X
White sucker, <i>Catostomus commersoni</i>	X	--	X	--	X	--
Bigmouth buffalo, <i>Ictiobus cyprinellus</i>	--	--	--	X	X	X
Spotted sucker, <i>Minytrema melanops</i>	--	--	--	X	--	X
White catfish, <i>Ictalurus catus</i>	--	--	X	--	X	--
Black bullhead, <i>Ictalurus melas</i>	--	--	X	X	X	X
Channel catfish, <i>Ictalurus punctatus</i>	X	X	X	X	X	--
Flathead catfish, <i>Pylodictis olivaris</i>	--	--	X	--	X	--
Brook stickleback, <i>Eucalia inconstans</i>	--	--	--	X	X	X
Green sunfish, <i>Lepomis cyanellus</i>	--	--	X	X	X	--
Pumpkinseed, <i>Lepomis gibbosus</i>	--	--	X	--	X	--
Bluegill, <i>Lepomis macrochirus</i>	--	--	X	X	X	--
Longear sunfish, <i>Lepomis megalotis</i>	--	--	X	--	X	--
Redear sunfish, <i>Lepomis microlophus</i>	--	--	X	--	X	--
Smallmouth bass, <i>Micropterus dolomieu</i>	--	--	X	--	X	--
Largemouth bass, <i>Micropterus salmoides</i>	--	X	X	X	X	--
Black crappie, <i>Pomoxis nigromaculatus</i>	--	--	X	X	X	--
Yellow perch, <i>Perca flavescens</i>	--	--	X	--	X	--
Walleye, <i>Stizostedion v. vitreum</i>	--	--	X	--	X	--
Freshwater drum, <i>Aplodinotus grunniens</i>	--	--	X	--	X	--

¹ May include juvenile as well as adult fish.

Fingerlings.--Routine bioassays were made with small fingerlings which usually range from 0.5 to 2.0 grams each. At least 10 fish of each species were used with each concentration of chemical and in each control. The loading in 5-gallon jars containing 15 liters of test solution or in 1-gallon jars containing 2.5 liters of solution was 1 gram or less of fish per liter. Observations on the responses of the fish to the toxicant were made at 3, 6, 24, 48, 72, and 96 hours.

Adults.--Since it is possible that the responses of adult fish to a toxicant may differ from those of younger fish, we exposed adults of 17 species to antimycin in 500-gallon concrete tanks, in 1,000-gallon vinyl tanks, or in 0.01-acre concrete pools. Most of them were obtained from the wild, and many of the tests were of the community type, involving two or more species at a time. All specimens were held in quarantine for several days to

determine whether they were in satisfactory condition for bioassays. They were stocked in the pools 3 to 7 days before exposure to antimycin.

TOXICITY

Effective concentrations.--Results of most of the bioassays of antimycin with fish are expressed as 24-, 48-, 72-, and 96-hour EC₀, EC₅₀, or EC₁₀₀, that is, the concentrations of toxicant in parts per billion which kill 0, 50, or 100 percent of the fish within 24, 48, 72, or 96 hours. In deriving the expressions, the responses observed in early tests were subjected to probit analyses to estimate the effective concentrations (Litchfield and Wilcoxon, 1949). The estimates were corrected or confirmed by subsequent bioassays with replications. Confidence intervals (C.I.) for EC₅₀'s are given where possible.

Effective contact time (ECT).--Antimycin kills fish slowly, and we suspect that specimens have had lethal exposures well before they showed signs of distress or death. Moreover, EC_{50} 's and EC_{100} 's at 24 through 96 hours in static bioassays do not pinpoint the minimum exposures necessary to kill fish. Nine species of fish were used in trials to define the durations of minimum lethal exposures in given concentrations of antimycin. Following the exposures at 12° C., the fish were transferred to fresh water and observed for at least 96 hours.

Toxicity of injected antimycin.--Six- to 12-inch rainbow trout, carp, and black bullheads were injected intraperitoneally with ethanol solutions of antimycin to determine toxicities which might possibly be compared with the immersional toxicities. The amounts of toxicant in milligrams were selected according to the body weights of the fish in kilograms. The injected volumes ranged from 0.2 to 1 ml. per fish. Control fish were given injections of ethanol in water, with the quantity of solvent equaling the greatest amounts used to dissolve the antimycin. The fish were held at 12° C. for 5 days, except that dead fish were discarded daily.

RESULTS

BIOASSAYS WITH FINGERLINGS

The majority of bioassays of antimycin in the laboratory in waters of various qualities involved small fingerlings. The responses of these fish, therefore, serve as standards by which the responses of other life stages are evaluated.

Temperature.--In general, fish are more susceptible to antimycin at warmer temperatures (table 3). There were twofold to fivefold differences between EC_{100} 's at 7° and 22° C.

Less than 1 p.p.b. of toxicant killed all individuals of 14 species at one or more temperatures. The more sensitive fish are rainbow trout, brown trout, brook trout, lake trout, and walleye. The often undesirable

carp, green sunfish, and pumpkinseed are also among the more sensitive fish. In contrast, the catfishes are relatively resistant. At temperatures from 22° to 7° C., 8 to 22 p.p.b. of antimycin are needed to kill channel catfish and 50 to 120 p.p.b. to kill black bullheads.

Water hardness.--Tests of antimycin against rainbow trout in waters of 20, 48, 90, 180, 360, and 400 p.p.m. total hardness demonstrated that the toxicant is slightly less effective in harder water. For example, 0.06 p.p.b. of the antibiotic killed all fish at 20 p.p.m. total hardness but none at 360 p.p.m.; and 0.08 p.p.b. killed less than half the fish at 360 p.p.m. total hardness.

In later tests, the 96-hour EC_{100} 's for rainbow trout, goldfish, and bluegill at 20 and 400 p.p.m. total hardness confirmed that antimycin is a little less effective in hard water.

pH.--The effectiveness of antimycin against fish is influenced substantially by the pH of the medium. Preliminary 96-hour bioassays were conducted with goldfish, at pH 5 to 10. The results indicated that EC_{100} 's increased three fold between pH 5 and 8; six fold between pH 8 and 9; and fourteen fold between pH 9 and 10. More definitive experiments with goldfish in 15-liter solutions of toxicant at pH 5 to 10 at 12° gave 96-hour EC_{100} 's of 0.20 p.p.b. at pH 5; 1.10 p.p.b. at pH 8; and 60 p.p.b. at pH 10 (table 4 and fig. 1).

Subsequently we attempted to conduct experiments with rainbow trout at various levels of pH, but the species were intolerant to the buffers at pH 6 and 10. The results at pH 7, 8, and 9 at 12° indicate that the toxicity of antimycin is reduced at higher pH levels. The 96-hour EC_{100} at pH 7 was 0.04 p.p.b., whereas it was 0.18 at pH 9.

A similar trend was evident in tests with carp at pH 7 through 10. The 96-hour EC_{100} 's of antimycin ranged from 0.6 p.p.b. at pH 7 to 4.0 p.p.b. at pH 9. At pH 10, there was a sharp increase in the EC_{100} to 20.0 p.p.b.

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TABLE 3.--Effective concentrations of antimycin on 22 species of fish in 96-hour exposures at selected temperatures

Species	Number of fish	Average size		Temperature (°C.)	Concentrations expressed in p.p.b.			
		Length (inches)	Weight (grams)		EC ₀	EC ₁₀₀	EC ₅₀ and 95-percent C.I.	
Shortnose gar.....	120	1.5	0.8	12	0.10	1.00	0.48	0.36- 0.65
Rainbow trout.....	180	--	1.5	7	0.06	0.20	0.08	0.07- 0.09
Do.....	180	--	1.5	12	0.02	0.60	0.04	0.03- 0.04
Do.....	204	--	1.6	17	0.01	0.04	0.03	0.02- 0.04
Brook trout.....	180	--	1.5	12	0.02	0.03	0.06	0.06- 0.07
Do.....	148	--	1.5	17	0.01	0.04	0.03	0.03- 0.04
Lake trout.....	60	3.3	4.0	12	0.06	0.10	0.07	0.06- 0.08
Northern pike.....	72	--	1.4	7	0.40	>0.60	0.55	0.50- 0.57
Do.....	176	--	0.8	12	0.10	0.40	0.26	0.23- 0.30
Do.....	176	--	2.0	17	0.08	0.20	0.14	0.12- 0.34
Do.....	168	--	1.9	22	<0.10	0.14	0.11	0.08- 0.16
Goldfish.....	192	--	2.3	7	0.20	2.00	1.00	0.83- 1.20
Do.....	200	--	2.0	12	0.20	1.00	0.50	0.42- 0.60
Do.....	192	--	2.0	17	0.10	0.40	0.35	0.25- 0.49
Do.....	216	1.2	0.4	22	0.10	0.40	0.20	0.16- 0.25
Northern redbelly dace	120	1.9	1.1	7	0.10	>0.50	0.52	0.37- 0.73
Do.....	120	1.9	1.1	12	<0.10	0.60	0.18	0.15- 0.21
Do.....	60	1.9	1.1	17	0.04	0.30	0.09	0.07- 0.12
Do.....	60	2.5	2.4	22	0.04	0.10	0.09	0.08- 0.11
Carp.....	120	--	2.0	7	0.20	0.80	0.43	0.36- 0.51
Do.....	240	--	2.0	12	0.10	0.60	0.35	0.30- 0.40
Do.....	120	--	2.0	17	0.10	0.40	0.25	0.21- 0.30
Do.....	96	--	2.0	22	0.04	0.20	0.12	0.09- 0.16
Fathead minnow.....	120	2.1	0.7	7	<0.20	0.40	0.20	0.17- 0.23
Do.....	120	2.1	1.6	12	0.08	0.40	0.21	0.16- 0.28
Do.....	120	2.1	0.7	17	0.06	0.12	0.09	0.08- 0.10
Do.....	120	2.1	1.7	22	0.04	0.08	0.06	0.05- 0.08
Black bullhead.....	120	2.3	2.4	7	40.00	120.00	88.00	73.00-105.00
Do.....	216	2.2	2.1	12	40.00	100.00	45.00	38.80- 52.20
Do.....	156	2.2	2.1	17	20.00	60.00	32.00	27.60- 37.20
Do.....	120	2.4	2.3	22	<20.00	50.00	21.00	15.50- 28.00
Channel catfish.....	120	--	1.9	7	6.00	22.00	10.50	9.50- 11.70
Do.....	120	--	1.9	12	4.00	16.00	9.00	7.30- 11.60
Do.....	180	--	1.9	17	2.00	>10.00	7.40	6.60- 8.40
Do.....	240	--	1.9	22	4.00	8.00	5.20	4.90- 5.60
Brook stickleback....	120	2.1	1.1	7	0.40	>0.60	0.55	0.52- 0.58
Do.....	180	2.1	1.1	12	0.10	0.40	0.21	0.18- 0.24
Do.....	120	2.1	1.1	17	0.10	0.25	0.16	0.14- 0.18
Do.....	120	2.1	1.1	22	<0.06	0.08	0.04	0.04- 0.05
Green sunfish.....	120	1.4	0.7	7	0.20	0.80	0.50	0.43- 0.59
Do.....	180	1.4	0.7	12	0.10	0.40	0.20	0.15- 0.24
Do.....	216	1.4	0.7	17	0.08	0.25	0.15	0.11- 0.18
Do.....	120	1.3	0.6	22	0.06	0.20	0.11	0.10- 0.12
Pumpkinseed.....	120	1.8	1.3	7	0.10	0.40	0.24	0.20- 0.29
Do.....	180	1.8	1.4	12	0.08	0.20	0.14	0.12- 0.17
Do.....	120	1.8	1.4	17	0.06	0.20	0.09	0.08- 0.10
Do.....	120	1.8	1.3	22	0.04	0.10	0.05	0.05- 0.07
Bluegill.....	120	--	2.5	7	0.20	0.60	0.50	0.45- 0.56
Do.....	120	--	2.5	12	0.08	0.20	0.14	0.11- 0.17
Do.....	180	--	1.3	17	0.06	0.15	0.07	0.06- 0.14
Do.....	180	--	0.8	22	0.04	0.10	0.06	0.05- 0.07
Longear sunfish.....	120	2.0	1.0	12	0.05	0.20	0.08	0.07- 0.11
Redear sunfish.....	120	1.8	1.3	17	0.05	0.25	0.09	0.08- 0.11
Smallmouth bass.....	120	1.3	0.5	12	0.01	0.10	0.04	0.03- 0.05
Do.....	120	1.3	0.5	17	0.02	0.08	0.04	0.03- 0.04
Do.....	120	1.3	0.5	22	0.01	0.12	0.06	0.04- 0.07
Largemouth bass.....	120	1.6	0.8	12	0.08	0.20	0.14	0.09- 0.20
Do.....	120	1.6	0.8	17	0.06	0.20	0.10	0.07- 0.10
Do.....	120	1.6	0.8	22	0.04	0.20	0.09	0.07- 0.11
Yellow perch.....	180	2.0	1.2	7	0.06	0.20	0.12	0.11- 0.14
Do.....	204	--	0.5	12	0.02	0.10	0.05	0.04- 0.06
Do.....	204	2.0	2.0	17	0.04	0.06	0.04	0.04- 0.05
Do.....	208	--	0.5	22	0.02	0.04	0.03	0.03- 0.04
Walleye.....	204	1.5	0.7	12	0.04	0.08	0.04	0.04- 0.05
Do.....	120	1.5	0.7	17	>0.01	0.03	0.02	0.02- 0.03
Freshwater drum.....	120	2.9	3.3	7	0.08	0.25	0.14	0.12- 0.17
Do.....	60	2.9	3.3	12	0.04	0.15	0.07	0.06- 0.09
Do.....	60	2.9	3.3	17	0.02	0.06	--	-- --
Do.....	60	2.9	3.3	22	0.01	0.04	0.02	0.01- 0.03

TABLE 4.--Concentrations of antimycin in p.p.b. which caused 0- and 100-percent mortality in fingerling rainbow trout, carp, and goldfish at pH levels 5 through 10 at 12° C.

pH	Number of fish	96-hour	
		EC ₀	EC ₁₀₀
<u>Rainbow trout</u>			
7 <u>±</u> 0.1.....	100	0.01	0.04
8 <u>±</u> 0.1.....	100	0.03	0.07
9 <u>±</u> 0.1.....	100	0.06	0.18
<u>Carp</u>			
7 <u>±</u> 0.1.....	100	0.10	0.60
8 <u>±</u> 0.1.....	100	0.10	1.00
9 <u>±</u> 0.1.....	100	1.60	4.00
10 <u>±</u> 0.1.....	100	9.40	20.00
<u>Goldfish</u>			
5 <u>±</u> 0.1.....	110	0.10	0.20
6 <u>±</u> 0.1.....	70	0.10	0.30
7 <u>±</u> 0.1.....	130	0.09	0.40
8 <u>±</u> 0.1.....	70	0.30	1.10
9 <u>±</u> 0.1.....	70	2.00	6.00
10 <u>±</u> 0.1.....	140	20.00	60.00

Turbidity.--The presence of 1,000 and 5,000 p.p.m. of clay in suspension influenced the toxicity of antimycin to 2.5-inch rainbow trout at 12° C. The medium with 1,000 p.p.m. of clay turbidity was only slightly less toxic than the control to the fish at 24, 48, and 96 hours. The high turbidity medium had 24-, 48-, and 96-hour EC₅₀'s of 0.40, 0.14, and 0.10 p.p.b., while those of the control were 0.16, 0.10, and 0.05 p.p.b.

Some additional observations on the performance of antimycin in turbid water are given under Simulated Field Trials.

Effective contact time.--Only brief exposures to antimycin are needed to kill fish (table 5). At 10 p.p.b. of toxicant, the ECT₁₀₀'s are 1 hour for green sunfish and bluegill, and 4 hours for carp, longear sunfish, and black crappie. In contrast, black bullheads require a minimum exposure of 3 to 4 hours to a concentration of 500 p.p.b. for a complete

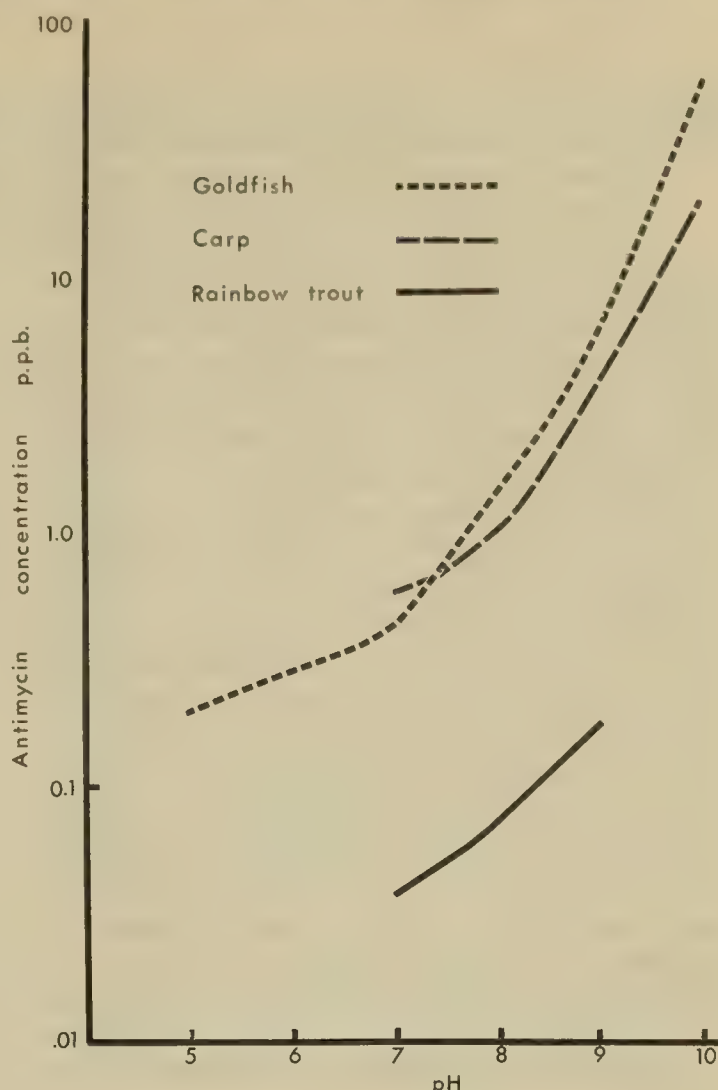


Figure 1.--Relation between various concentrations of antimycin and levels of pH necessary to produce EC₁₀₀'s for selected fishes at 12° C.

kill. These data are especially pertinent if the toxicant were to be used in the reclamation of streams or in situations where high pH contributes to rapid degradation.

Sand-formulated toxicant.--Preparations of antimycin coated on sand were obtained rather late in the experimental program. Fintrol-5 and Fintrol-15 were compared with crystalline antimycin in acetone solutions for effectiveness against fingerling rainbow trout and carp.

Fintrol-5 contains 10 mg. of antimycin per gram of formulation. Its 96-hour EC₅₀'s for rainbow trout and carp at 12° C. were 0.04 and 0.20 p.p.b. respectively. By comparison, the EC₅₀'s of acetone-antimycin under the same circumstances were rainbow trout 0.03 p.p.b. and carp 0.20 p.p.b.

TABLE 5.--Effective contact time (ECT) of antimycin against selected fishes at 12°C in standard reconstituted water¹

Species	Number of fish	Average size		Antimycin (p.p.b.)	ECT ₀	ECT ₅₀	ECT ₁₀₀
		Length (in.)	Weight (g.)				
Goldfish.....	50	1.5	1.0	50	< 15 min.	15-30 min.	30-60 min.
Carp.....	80	1.6	0.9	1	< 2 hrs.	2-4 hrs.	> 4 hrs.
Do.....	80	1.6	0.9	10	30-60 min.	1-2 hrs.	2-4 hrs.
Do.....	40	1.6	0.9	50	< 7.5 min.	7.5-15 min.	15-30 min.
Do.....	80	1.6	0.9	100	< 4 min.	4-7.5 min.	7.5-15 min.
Redbelly dace.....	80	1.9	1.1	1	4 hrs.	7.5 hrs.	12-14 hrs.
Do.....	80	1.9	1.1	10	3.0-7.5 min.	30-45 min.	2 hrs.
Black bullhead.....	80	2.2	2.2	250	< 4 hrs.	> 8 hrs.	> 8 hrs.
Do.....	80	2.2	2.2	500	30-60 min.	1-2 hrs.	3-4 hrs.
Do.....	80	2.2	2.2	1,000	15-30 min.	1-2 hrs.	2-4 hrs.
Green sunfish.....	80	1.4	1.0	1	< 6 hrs.	> 6.5 hrs.	22 hrs.
Do.....	80	1.4	1.0	10	< 15 min.	15 min.	30-60 min.
Do.....	80	1.4	1.0	100	< 1 min.	1.5 min.	> 4 min.
Bluegill.....	80	1.7	1.1	1	> 15 min.	15-30 min.	30-60 min.
Do.....	80	1.7	1.1	10	> 7.5 min.	7.5-15 min.	15-60 min.
Do.....	80	1.7	1.1	50	1-2 min.	2-4 min.	4-7.5 min.
Do.....	80	1.7	1.1	100	1-2 min.	2-4 min.	4-7.5 min.
Longear sunfish.....	60	3.1	7.8	10	15-30 min.	1-2 hrs.	2-4 hrs.
Do.....	60	3.1	7.8	100	1-2 min.	2-7.5 min.	7.5-15 min.
Black crappie.....	35	3.6	7.8	1	30-60 min.	> 4 hrs.	> 4 hrs.
Do.....	35	3.6	7.8	10	4-7.5 min.	0.5-2 hrs.	2-4 hrs.
Do.....	35	3.6	7.8	100	1-2 min.	7.5-15 min.	15-30 min.
Yellow perch.....	80	2.2	1.6	1	30 min.	1.0-1.5 hrs.	> 1.5 hrs.
Do.....	80	2.2	1.6	10	< 30 min.	30 min.	1-2 hrs.
Do.....	80	2.2	1.6	100	< 15 min.	< 15 min.	< 15 min.

¹ All test animals were observed for a minimum of 96 hours after transfer to fresh water.

Fintrol-15 has 26 mg. of antimycin per gram of formulation. Its EC₅₀'s at 96 hours of 0.07 p.p.b. for rainbow trout and 0.15 p.p.b. for carp compare very favorably with those of acetone-antimycin or Fintrol-5 solutions.

Column tests.--The release of antimycin from Fintrol-5 is apparently uniform but not complete within the first 5 feet of depth in the 8-foot plexiglass column. Samples siphoned at depths of 1 to 5 feet were toxic to all rainbow trout within 24 hours. Four of 16 trout survived in a sample taken at 6 feet, and all trout lived in a sample at 7 feet. There is obviously some further release of toxicant from sand lying on the bottom, because a sample of water drawn at 8 feet killed all trout.

We were unable to test the release of antimycin from Fintrol-15 at depths greater than 8 feet. The bioassays of samples taken at 1 through 8 feet in the column demonstrated a uniform release of toxicant.

Some of the first samples of Fintrol had a tendency to lie on the surface film when sprinkled lightly at the top of the column. This was corrected by the manufacturer, and later samples penetrated the film, sank readily to the bottom, and gave a good dispersion of the toxicant.

BIOASSAYS WITH OTHER LIFE STAGES

Eggs.--Antimycin is toxic to fertilized eggs of rainbow trout, northern pike, goldfish, carp, white sucker, and channel catfish. Recently, Valentine (1966) demonstrated that the antibiotic also kills fertilized eggs of zebra fish (*Brachydanio rerio*).

The first experiments included newly fertilized, water-hardened eggs of rainbow trout which had developed to about the 32-cell stage during the first 24 hours. Groups of 100 eggs were exposed to 1 and 100 p.p.b. of antimycin for 30, 60, and 120 minutes

and then transferred into a Heath egg incubator. Control groups were handled similarly but not exposed to the toxicant. Embryogeny was compared by removing several eggs per day from treated and untreated groups. After 2 weeks, the eggs were sampled each 2 to 4 days. The samples were fixed in 5-percent formalin in 1-percent saline. Chorions were removed, and the embryos were stained in Harris hematoxylin and examined.

Within 24 hours, the eggs exposed to 100 p.p.b. of toxicant for 120 minutes ceased development at about the 64-cell stage. Among control eggs, the blastoderms were well developed. Coagulation of protein was evident in dead eggs on the fourth day after treatment, and all eggs in the group were opaque by the seventh day.

The eggs exposed for only 60 minutes to 100 p.p.b. of toxicant developed somewhat further. Cell division in some continued for 2 days. Protein coagulation in dead eggs continued from the fourth to the 11th day.

Some eggs exposed to 100 p.p.b. of antimycin for 30 minutes showed development comparable to that of controls for 8 to 13 days after treatment. In others, development terminated at the blastoderm stage. The greater number of opaque eggs appeared after 7 days. Microscopic examination of the embryos revealed no gross morphological or anatomical changes attributable to the toxicant.

The eggs exposed to 1 p.p.b. of toxicant and control eggs were "eyed" 15 days after fertilization, and they hatched 16 days later. There was no significant difference in the hatching success of the treated and control groups.

Long-term exposures to small concentrations of antimycin effected eggs of rainbow trout. Acetone solutions or sand formulations of toxicant were added to 1-gallon jars which contained approximately 100 fertilized eggs each. The medium was reconstituted water at 12° C., pH 7.2 to 7.6, and total hardness of 40 p.p.m. The eggs remained in the jars throughout incubation and swim-up.

The eggs became eyed after 15 days and hatched at 29 days, and the sac fry reached swim-up stage at 42 days. Observations continued through 67 days, at which time the control fish were of routine bioassay size, that is, approximately 500 per pound. None of the eggs exposed to 0.5 p.p.b. or more of acetone-antimycin survived to the eyed stage (table 6). Most embryos from eggs exposed to 0.1 p.p.b. lived through the eyed and hatching stages, but mortalities were heavy at swim-up, as the fry seemed unable to take food. Only 7 percent survived through 67 days. All fry from eggs exposed to the sand formulation at 0.1 to 1.0 p.p.b. of antimycin perished within 42 days. Eggs treated with acetone-antimycin solutions survived through 67 days. The control group had a survival of 55 percent at 67 days. Bioassays with fingerling rainbow trout in eggless control solutions of the toxicant demonstrated that both formulations had degraded within the first 14 days of the experiment.

In another series of tests at 12°, the eggs of northern pike exhibited greater sensitivity to antimycin than those of rainbow trout and white sucker (table 7). The green eggs of trout were more sensitive than eyed eggs.

The eggs of goldfish and carp also proved susceptible to antimycin. All goldfish eggs were killed by 2-hour exposures to 7.5 and 10 p.p.b. of toxicant, and only 1 percent survived 5 p.p.b. (table 8). All eggs of goldfish and carp died when exposed to 2.5 p.p.b.

TABLE 6.--Mortality of rainbow trout eggs incubated in solutions of antimycin at 12° C.

Antimycin	Number of eggs	Percent mortality at--					
		3 days	6 days	15 days ¹	29 days ²	42 days ³	67 days ⁴
In acetone:							
0.10 p.p.b.	107	9	13	30	37	87	93
0.25 p.p.b.	125	10	36	91	93	96	100
0.50 p.p.b.	116	9	100	--	--	--	--
0.75 p.p.b.	105	10	100	--	--	--	--
1.00 p.p.b.	111	12	100	--	--	--	--
On sand:							
0.10 p.p.b.	97	6	7	9	20	100	--
0.25 p.p.b.	90	6	8	18	27	100	--
0.50 p.p.b.	98	5	82	100	--	--	--
0.75 p.p.b.	88	5	76	100	--	--	--
1.00 p.p.b.	101	5	100	--	--	--	--
Control.....	105	4	6	10	35	40	45

¹ Eggs eyed.

² Eggs hatched.

³ Swim-up.

⁴ Fingerling stage.

TABLE 7.--Survival of fish eggs after exposure to aqueous solutions of antimycin in acetone at 12° C.

[The postexposure period of observation was 3 to 30 days]

Species and age of eggs	Number of eggs	Length of exposure (min.)	Percentage survival at concentrations (in p.p.b.)		
			1.0	10.0	100.0
Rainbow trout (24 hours after fertilization)	300	30	100	20	0
	300	60	100	0	0
Rainbow trout (3 days before hatching)	300	30	100	100	0
	300	60	100	100	0
	300	120	100	0	0
Northern pike (24 hours after fertilization) ¹	600	30	100	100	0
	600	60	80	50	0
	600	120	25	0	0
White sucker (8 days before hatching)	450	30	100	100	0
	450	60	100	100	0
	450	120	100	0	0

¹ Not feasible to hold 30 days.

TABLE 8.--Survival of 24-hour-old eggs of goldfish after 2-hour exposure to antimycin at 17° C.

Antimycin	Number of eggs	Percentage hatch
1.0 p.p.b.....	100	47
2.5 p.p.b.....	100	15
5.0 p.p.b.....	100	1
7.5 p.p.b.....	100	0
10.0 p.p.b.....	100	0
Control.....	100	61

or more throughout the 7-day incubation period. Further experiments at 19° to 23° showed that 10 p.p.b. of antimycin which were allowed to degrade for 96 hours were no longer toxic to goldfish eggs. However, a solution which originally contained 20 p.p.b. of toxicant killed 70 percent of exposed goldfish eggs within 96 hours.

The eggs of channel catfish can be killed with antimycin (table 9), but they are substantially more resistant than those of rainbow trout, northern pike, goldfish, carp, and white sucker. Fertilized eggs about 24 hours old were placed in small baskets of saran mesh. Some were exposed for 2 hours to selected concentrations of antimycin; others were exposed to the toxicant throughout the 6-day incubation period. The solutions of toxicant were aerated, constantly agitated, and temperatures ranged between 25° and 28.5° C. At 100 p.p.b. or less, over 90 percent of the eggs survived 2-hour exposures.

TABLE 9.--Survival of eggs of channel catfish following brief and prolonged exposures to antimycin at 25° to 28.5° C.

Antimycin	Number of eggs	Percentage hatch
Two-hour exposure:		
20 p.p.b.....	100	93
40 p.p.b.....	100	96
60 p.p.b.....	100	93
80 p.p.b.....	100	100
100 p.p.b.....	200	97
250 p.p.b.....	100	19
500 p.p.b.....	100	0
750 p.p.b.....	100	0
1,000 p.p.b.....	100	0
Control.....	200	95
Six-day exposures:		
10.0 p.p.b.....	30	93
12.5 p.p.b.....	30	96
15.0 p.p.b.....	30	83
17.5 p.p.b.....	30	90
20.0 p.p.b.....	60	95
25.0 p.p.b.....	30	26
27.5 p.p.b.....	30	0
30.0 p.p.b.....	30	0
40.0 p.p.b.....	30	0
50.0 p.p.b.....	30	0
Control.....	60	96

In contrast, all eggs succumbed to prolonged exposures to 27.5 p.p.b. or more of antimycin.

Fry.--Bowfin, rainbow trout, channel catfish, and largemouth bass fry were subjected to antimycin. Because their ages differed, we cannot say which species are the more sensitive at a given age.

Bowfin exhibited increasing resistance to the toxicant with increasing age. The 96-hour EC₅₀'s were 0.13 p.p.b. at 3 weeks of age, 0.24 p.p.b. at 6 weeks, and 0.35 p.p.b. at 8 weeks. A comparable trial with 15 p.p.b. killed 12 adult fish.

The responses of rainbow trout sac fry and advanced fry to antimycin were approximately the same as those of fingerlings (table 10). The 96-hour EC₅₀'s of 5-, 12-, and 18-day-old sac fry at 12° C. were 0.04, 0.03, and 0.05 p.p.b. respectively, whereas the EC₅₀ for 44-day-old advanced fry and 60-day-old fingerlings was 0.04 p.p.b.

The fry of channel catfish, only a few hours old, were more sensitive than the eggs to the toxicant. On the other hand, they were more

TABLE 10.--Toxicity of antimycin in p.p.b. to sac and advanced fry of rainbow trout at 12° C.

Age	Number of fish	24-hour				96-hour			
		EC ₀	EC ₁₀₀	EC ₅₀	C.I. ¹	EC ₀	EC ₁₀₀	EC ₅₀	C.I. ¹
3 days....	100	0.10	0.40	0.30	.12-.42	--	--	--	--
5 days....	100	0.10	0.60	0.35	.28-.34	0.02	0.08	0.04	.02-.05
11 days....	100	0.06	0.20	0.14	.12-.18	--	--	--	--
12 days....	100	0.06	0.20	0.15	.10-.22	0.02	0.04	0.03	.02-.04
14 days....	100	0.04	0.10	--	--	--	--	--	--
18 days....	100	0.06	0.40	--	--	0.02	0.08	0.05	.03-.06
25 days....	100	0.10	0.40	0.32	.30-.34	0.04	0.08	--	--
28 days....	100	0.04	0.20	0.12	.09-.14	--	--	--	--
44 days....	100	0.08	0.40	0.27	.24-.30	0.02	0.06	0.04	.02-.05
60 days ² ...	100	0.10	0.60	0.45	.36-.46	0.02	0.06	0.04	.03-.05

¹ C.I. = 95-percent confidence intervals for EC₅₀'s² Approximate age of our standard bioassay fish

resistant than the fry of bowfin and rainbow trout. In standard bioassays at 22° C., the 96-hour EC₅₀ was 1 p.p.b. For those fry exposed to the toxicant for only 2 hours at 26° C., the EC₅₀ was 26.9 p.p.b. and the EC₁₀₀ was 50 p.p.b.

Newly hatched fry of largemouth bass also were exposed to antimycin for a 2-hour period at concentrations of 0.01 to 0.50 p.p.b. Many of the control fry died, however, which makes interpretation of the results difficult. There was a 100-percent mortality of fry exposed to 0.075 p.p.b. within the following 96 hours, but there was a 60-percent mortality during the same period in the control lot of fry. Eighty-two of the 100 fry exposed to the lowest concentration of antimycin, 0.01 p.p.b., died within the following 96 hours.

Juveniles and adults.--Most of the tests of antimycin against larger fish were run in vinyl or concrete pools. An early series in 1,000-gallon, vinyl pools at 15.5° to 17.2° C., pH 8.4 to 8.6, and total hardness of 252 p.p.m. included 10 species of fish. Five p.p.b. or less of toxicant killed all goldfish, carp, fathead minnow, bigmouth buffalo, green sunfish, pumpkinseed, bluegill, and largemouth bass within 144 hours (table 11). Most black bullheads and some channel catfish survived exposures to 10 and 20 p.p.b. Naiads of damselflies and aquatic plants in the pools were unharmed by the antimycin. Subsequent restocking with small bluegills indicated that the toxicant had degraded within the first 144 hours.

Followup bioassays in vinyl pools determined the concentration of antimycin necessary to kill black bullheads. Included were 40, 80, 120, 160, and 200 p.p.b. of toxicant at 26.6° C., pH 9.0 to 9.6 and five species of fish. The 3.6-inch goldfish, 2.8-inch carp, 2.7-inch green sunfish, and 4.9-inch bluegills were killed within 24 hours by all concentrations. The 5-inch wild black bullheads exhibited stress within 3 hours at 160 and 200 p.p.b., but they recovered fully within 24 hours. At these relatively high levels of pH, the toxicant degraded within 48 hours.

In contrast, 5-inch black bullheads alone in a test at 23° C., pH 7.8 to 8.6, and 287 p.p.m. total hardness, died within 24 hours in 160 and 200 p.p.b. of antimycin. None died at lower concentrations. The toxicant lasted longer at this range of pH, and degradation was not considered complete until 96 hours.

Fifteen-inch white catfish and 6-inch green sunfish were exposed up to 144 hours in 1, 5, and 25 p.p.b. of acetone- or sand-formulated antimycin in vinyl pools at temperatures which ranged between 1° and 16° C., pH 6.2 to 7.0, and total hardness of 18 to 40 p.p.m. All white catfish survived, but the green sunfish died in either formulation at 5 p.p.b. The average degradation time for the acetone formulation was a little more than 7 days; for the sand formulation it was slightly less than that.

A separate series of tests was performed with acetone-antimycin against large fingerling

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TABLE 11.--Toxicity of antimycin to various species and sizes of fish in outdoor, vinyl pools at 15.5° to 17.2° C., pH 8.4 to 8.6, and 252 p.p.m. total hardness

Species	Number of fish	Average size		Antimycin (p.p.b.)	Fish mortality at-- ¹						Totals		
		Length (in.)	Weight (g.)		6 hours	24 hours	48 hours	72 hours	96 hours	144 hours	Alive	Dead	Lost
Goldfish.....	30	2.5	4.2	Control	0	0	0	0	0	0	10	0	² 20
Do.....	30	"	"	0.5	0	0	0	0	0	P	17	1	12
Do.....	30	"	"	1.0	0	0	0	0	0	P	17	1	12
Do.....	30	"	"	5.0	0	0	0	P	P	P	0	20	10
Do.....	30	"	"	10.0	0	P	P	P	P	P	0	16	14
Do.....	30	"	"	20.0	0	P	P	P	P	P	0	17	13
Do.....	30	4.1	24.1	Control	0	0	0	0	0	0	26	0	4
Do.....	30	"	"	0.5	0	0	0	0	0	P	20	1	9
Do.....	30	"	"	1.0	0	0	0	0	0	P	21	1	8
Do.....	30	"	"	5.0	0	0	P	P	P	P	0	26	4
Do.....	30	"	"	10.0	0	P	P	P	P	P	0	29	1
Do.....	30	"	"	20.0	0	P	P	P	P	P	0	27	3
Carp.....	30	2.2	2.5	Control	0	0	0	P	P	P	12	2	16
Do.....	30	"	"	0.5	0	P	P	P	P	P	5	17	8
Do.....	30	"	"	1.0	0	P	P	P	P	T	0	30	0
Do.....	30	"	"	5.0	P	T	T	T	T	T	0	30	0
Do.....	30	"	"	10.0	T	T	T	T	T	T	0	30	0
Do.....	30	"	"	20.0	P	T	T	T	T	T	0	29	1
Do.....	30	6.4	56.4	Control	0	0	0	0	0	0	30	0	0
Do.....	30	"	"	0.5	0	0	0	0	0	0	30	0	0
Do.....	30	"	"	1.0	0	0	0	0	0	P	22	6	2
Do.....	30	"	"	5.0	0	P	P	P	P	T	0	30	0
Do.....	30	"	"	10.0	P	P	P	P	T	T	0	30	0
Do.....	30	"	"	20.0	0	P	T	T	T	T	0	30	0
Fathead minnow.....	30	2.2	4.0	Control	0	0	0	P	P	P	28	2	0
Do.....	30	"	"	0.5	0	P	P	P	P	T	0	30	0
Do.....	30	"	"	1.0	0	P	P	T	T	T	0	30	0
Do.....	30	"	"	5.0	P	T	T	T	T	T	0	30	0
Do.....	30	"	"	10.0	P	T	T	T	T	T	0	30	0
Do.....	30	"	"	20.0	T	T	T	T	T	T	0	30	0
Bigmouth buffalo.....	20	7.2	102.0	Control	0	0	0	0	0	0	18	1	1
Do.....	20	"	"	0.5	0	0	0	0	0	0	19	1	0
Do.....	20	"	"	1.0	0	0	0	0	0	P	12	1	7
Do.....	20	"	"	5.0	0	P	P	P	P	T	0	20	0
Do.....	20	"	"	10.0	0	P	T	T	T	T	0	19	1
Do.....	20	"	"	20.0	0	P	T	T	T	T	0	17	3
Black bullhead.....	30	2.3	2.6	Control	0	0	0	0	0	0	16	0	14
Do.....	30	"	"	0.5	0	0	0	0	0	0	14	0	16
Do.....	30	"	"	1.0	0	0	0	0	0	0	20	0	10
Do.....	30	"	"	5.0	0	0	0	0	0	0	18	1	11
Do.....	30	"	"	10.0	0	0	0	0	0	0	18	1	11
Do.....	30	"	"	20.0	0	0	0	0	0	0	21	0	9
Black bullhead.....	30	5.7	30.1	Control	0	0	0	0	0	0	27	0	3
Do.....	30	"	"	0.5	0	0	0	0	0	0	28	0	2
Do.....	30	"	"	1.0	0	0	0	0	0	0	25	0	5
Do.....	30	"	"	5.0	0	0	0	0	0	0	25	0	5
Do.....	30	"	"	10.0	0	0	0	0	0	0	30	0	0
Do.....	30	"	"	20.0	0	0	0	0	0	0	30	0	0
Channel catfish.....	30	2.4	1.8	Control	0	0	P	P	P	P	19	11	0
Do.....	30	"	"	0.5	0	0	0	0	0	P	1	20	9
Do.....	30	"	"	1.0	0	0	0	0	0	P	4	26	0
Do.....	30	"	"	5.0	0	0	0	0	0	0	17	13	0
Do.....	30	"	"	10.0	0	0	0	0	0	0	13	15	2
Do.....	30	"	"	20.0	P	P	P	P	P	P	2	28	0
Green sunfish.....	30	1.5	1.0	Control	0	0	0	0	0	0	30	0	0
Do.....	30	"	"	0.5	0	0	P	P	P	T	0	30	0
Do.....	30	"	"	1.0	0	P	P	T	T	T	0	30	0
Do.....	30	"	"	5.0	P	P	T	T	T	T	0	30	0
Do.....	30	"	"	10.0	P	T	T	T	T	T	0	30	0
Do.....	30	"	"	20.0	T	T	T	T	T	T	0	30	0
Green sunfish.....	30	2.8	5.4	Control	0	0	0	0	0	0	27	0	3
Do.....	30	"	"	0.5	0	0	0	P	P	P	15	12	3
Do.....	30	"	"	1.0	0	0	P	P	P	P	0	17	13
Do.....	30	"	"	5.0	P	P	P	P	P	P	0	18	12
Do.....	30	"	"	10.0	P	P	P	P	T	T	0	18	12
Do.....	30	"	"	20.0	P	P	P	T	T	T	0	20	10
Pumpkinseed.....	30	3.0	8.4	Control	0	0	0	0	0	0	24	0	6
Do.....	30	"	"	0.5	0	0	0	0	P	P	7	13	10
Do.....	30	"	"	1.0	0	0	0	P	P	P	0	26	4
Do.....	30	"	"	5.0	0	0	P	P	P	P	0	30	0
Do.....	30	"	"	10.0	0	P	P	P	P	T	0	26	4
Do.....	30	"	"	20.0	0	P	P	P	P	T	0	25	5

See footnotes at end of table.

TABLE 11.--Toxicity of antimycin to various species and sizes of fish in outdoor, vinyl pools at 15.5° to 17.2° C., pH 8.4 to 8.6, and 252 p.p.m. total hardness--continued

Species	Number of fish	Average size		Antimycin (p.p.b.)	Fish mortality at-- ¹						Totals		
		Length (in.)	Weight (g.)		6 hours	24 hours	48 hours	72 hours	96 hours	144 hours	Alive	Dead	Lost
Bluegill.....	30	1.6	0.8	Control	O	O	O	O	O	O	30	0	0
Do.....	30	"	"	0.5	O	P	T	T	T	T	0	30	0
Do.....	30	"	"	1.0	O	P	T	T	T	T	0	30	0
Do.....	30	"	"	5.0	P	T	T	T	T	T	0	30	0
Do.....	30	"	"	10.0	P	T	T	T	T	T	0	30	0
Do.....	30	"	"	20.0	P	T	T	T	T	T	0	30	0
Bluegill.....	30	5.3	50.0	Control	O	P	P	P	P	P	9	10	11
Do.....	30	"	"	0.5	O	P	P	P	P	P	0	23	7
Do.....	30	"	"	1.0	O	P	P	P	P	P	0	26	4
Do.....	30	"	"	5.0	O	P	P	P	P	T	0	25	5
Do.....	30	"	"	10.0	O	P	P	P	P	T	0	20	10
Do.....	30	"	"	20.0	P	P	P	P	T	T	0	25	5
Largemouth bass.....	30	3.0	5.2	Control	O	O	O	O	P	P	0	20	10
Do.....	30	"	"	0.5	O	O	P	P	P	P	11	19	0
Do.....	30	"	"	1.0	P	P	P	P	T	T	0	30	0
Do.....	30	"	"	5.0	P	P	T	T	T	T	0	30	0
Do.....	30	"	"	10.0	P	T	T	T	T	T	0	30	0
Do.....	30	"	"	20.0	T	T	T	T	T	T	0	30	0
Largemouth bass.....	10	7.2	81.0	Control	O	O	O	O	O	O	9	0	1
Do.....	10	"	"	0.5	O	O	O	P	P	P	6	4	0
Do.....	10	"	"	1.0	O	O	O	P	P	P	0	8	2
Do.....	10	"	"	5.0	O	O	P	P	P	P	0	10	0
Do.....	10	"	"	10.0	O	O	P	P	P	T	0	10	0
Do.....	10	"	"	20.0	O	P	P	P	P	T	0	8	2

¹ O = no mortality, P = partial mortality, T = total mortality.² Unaccountable loss from pools, due in part to predatory birds.

flathead catfish in vinyl pools which contained water of 42 to 48 p.p.m. in total hardness and pH 6.6 to 7.0. One group of 4.5-inch fish survived 96-hour exposures to 50 p.p.b. of antimycin at 21° C. The 96-hour EC₅₀ for a group of 5.8-inch flatheads was 54 p.p.b. at 27° C. In a test at 28° C., the 24-, 48-, and 72-hour EC₅₀'s for 5.8-inch fingerlings was 33 p.p.b. Most of the deaths at 27 and 28° C. were within the first 6 hours of the test. In a separate experiment, the 96-hour EC₅₀ for adult flathead catfish at 17° C. was 182 p.p.b. (95-percent C.I. = 158-209).

Eight species of fingerling and larger fish were exposed to 0.1, 1.0, and 5.0 p.p.b. of antimycin under ice cover. The sand formulation was applied in vinyl pools containing water at 190 and 282 p.p.m. total hardness and pH 8.5 to 9.0. The activity of the toxicant was influenced far more by the temperature under 5 to 6 inches of ice than by water quality. Concentrations of 0.1 and 1.0 p.p.b. caused no significant mortality of fish. Five p.p.b. of toxicant killed 5.8-inch rainbow trout and 5.9-inch brown trout within 72 hours, and 1.6-inch carp and 3.1-inch longear sunfish within 120 hours (table 12). There were some deaths among 1.4-inch goldfish, 2.1-inch fathead minnow, and 1.4- and 2.8-inch bluegill. No deaths occurred among 3.8-inch goldfish, 7.2-

inch carp, and 2.6-inch pumpkinseeds. At the relatively high pH, the toxicant degraded to a point harmless to brown trout within 120 hours after application.

Some tests with adults of resistant species were made indoors in concrete tanks with well water at total hardness of 300 p.p.m., pH 7.6, and 14.5° C. Some of the shortnose gar died at 15 and 20 p.p.b. of sand-formulated antimycin, but 25 p.p.b. were required for complete kills within 96 hours (table 13). All bowfin died at 20 p.p.b. In sharp contrast, black bullheads showed 50-percent mortality at 140 p.p.b. and 100-percent died at 220 p.p.b. Their resistance to the toxicant, therefore, is at least 10 times that of gar and bowfin.

SIMULATED FIELD TRIALS

Community bioassays with juvenile and adult fish in 0.01-acre concrete pools provided further evidence on the concentrations of antimycin needed to kill certain species. The pools were 3.5 feet deep, the water temperatures were 11° to 21° C., pH ranged from 7.9 to 8.6, total hardness from 252 to 290 p.p.m., and total alkalinity from 211 to 250 p.p.m. The concentrations of dissolved oxygen ranged from 8.1 to 11.2 p.p.m. Under these

TABLE 12.--Toxicity of 5 p.p.b. of antimycin in sand formulation to fish in vinyl pools under 5 to 6 inches of ice cover, total hardnesses of 190 and 282 p.p.m. and pH 8.5 to 9.0

Species	Number of fish per pool	Average size		Cumulative kill (hrs.)						Cumulative kill (hrs.)					
		Length (in.)	Weight (g.)	Hardness of 190 p.p.m.						Hardness of 282 p.p.m.					
				6	24	48	72	96	120	6	24	48	72	96	120
Rainbow trout.....	10	5.8	32.0	0	1	9	10	--	--	0	2	10	--	--	--
Brown trout.....	10	5.9	38.0	0	0	3	10	--	--	0	0	5	10	--	--
Goldfish.....	10	1.4	0.7	0	2	1	5	5	6	0	0	0	5	9	9
Do.....	10	3.8	22.8	0	0	0	0	0	0	0	0	0	0	0	0
Carp.....	10	1.6	0.9	0	0	3	3	9	10	0	0	1	6	10	--
Do.....	10	7.2	82.2	0	0	0	0	0	0	0	0	0	0	0	0
Fathead minnow.....	10	2.1	1.6	0	0	0	0	0	0	0	0	0	0	0	1
Bluegill.....	20	1.4	0.7	0	0	2	10	15	16	0	0	8	16	20	--
Do.....	10	2.8	5.2	0	0	0	1	1	1	0	0	0	0	1	3
Pumpkinseed.....	10	2.6	6.0	0	0	0	0	0	0	0	0	0	0	0	0
Longear sunfish.....	10	3.1	8.0	0	0	2	3	9	10	0	0	1	5	10	--

TABLE 13.--Toxicity of sand-formulated antimycin to adults of three resistant species in 96 hours at 14.5° C.

Species	Number per concentration	Length (in.)		Percent mortality at concentrations of (p.p.b.) ¹											
		Average	Range	10	15	20	25	90	120	140	180	220			
Shortnose gar.....	5	23.0	20.0-26.0	0	20	80	100	--	--	--	--	--			
Bowfin.....	5	22.0	16.0-24.0	20	60	100	100	--	--	--	--	--			
Black bullhead.....	50	6.5	5.5- 9.0	--	--	--	--	0	14	50	74	100			

¹ There was no mortality among controls of each species.

conditions, 1 p.p.b. of sand-formulated antimycin killed all rainbow trout and 86 percent of the bluegills but failed to affect the two size groups of goldfish and the carp (table 14). Two p.p.b. killed all spotted suckers and green sunfish as well as the trout and bluegills. Four p.p.b. was the lowest concentration at which all carp perished, but a small number of the larger goldfish survived both 4 and 5 p.p.b. All fish of the 12 species were eradicated by 10 p.p.b.

Caged rainbow trout of fingerling size were placed in the pools each 24 hours. Their survival showed that the toxicant degraded to a safe level for the species within 8 days in every test in all pools.

A cold-weather test in the 0.01-acre pools demonstrated that Fintrol-5 is effective against fish in cold, shallow, clear, or turbid waters. The pools were maintained at 2 feet deep during the 3-week trial. Water temperatures ranged from 3.4° to 5.5° C., pH from 7.4 to

8.2, total hardness from 260 to 320 p.p.m., and alkalinity from 235 to 260 p.p.m. The Secchi disk reading was 6 inches in four turbid pools and to the bottom in four clear pools.

Juvenile or adult brown trout, carp, bluegill, and largemouth bass in clear pools succumbed within 2 to 7 days to 5 p.p.b. of antimycin and within 1 to 3 days at 10 p.p.b. Death was delayed in turbid water; the fish died within 7 to 12 days at 5 p.p.b. and within 3 to 7 days at 10 p.p.b. Large goldfish were the most resistant fish, but all were killed by 5 p.p.b. of antimycin within 15 days in clear water and within 18 days in turbid water; the kill at 10 p.p.b. occurred within 11 days in clear water and within 16 days in turbid water.

Fingerling yellow perch in live cages were used to detect the degradation of Fintrol-5 in the pools. The process was slow in the cold water, but degradation occurred sooner

TABLE 14.--Toxicity of sand-formulated antimycin to juveniles or adults of 12 species of fish in 0.01-acre pools

Species	Number per concentration	Length (in.)		Percent mortality at concentrations of (p.p.b.)							
		Average	Range	1	2	3	4	5	10	20	Control
Rainbow trout.....	10	9.9	8.8-11.0	100	100	100	100	100	100	100	0
Brown trout.....	20	7.5	6.5- 8.3	--	--	--	--	--	100	100	0
Lake trout.....	20	4.7	3.5- 5.2	--	--	--	--	--	100	100	0
Northern pike.....	10	20.0	12.0-22.5	10	50	100	100	100	100	100	20
Goldfish.....	30	3.8	3.0- 5.0	0	30	70	--	100	100	100	10
Do.....	10	6.9	4.8- 8.1	0	0	30	80	90	100	100	0
Carp.....	35	12.1	7.4-22.0	0	33	96	100	100	100	100	3
Quillback.....	8	13.9	8.0-16.0	--	--	100	--	100	--	--	0
Spotted sucker.....	6	14.8	13.8-15.7	--	100	--	100	--	--	--	0
Green sunfish.....	40	3.6	2.8- 4.4	--	100	100	--	--	--	--	10
Bluegill.....	30	6.0	4.2- 7.8	86	100	100	100	100	100	100	5
Largemouth bass.....	10	7.6	5.4- 8.6	--	50	100	100	100	--	--	0
Black crappie.....	35	9.8	8.0-11.0	43	--	--	--	--	100	100	20

in clear water than in turbid water. Five and 10 p.p.b. of antimycin disappeared within 8 and 13 days respectively in clear water and within 9 and 17 days in turbid water.

Water temperature has greater influence than turbidity on the response of fish to antimycin, but mortality of the fish is merely retarded rather than reduced in cold and turbid water.

TOXICITY OF INJECTED ANTIMYCIN

Injections of antimycin into the peritoneal cavities of fish are toxic, and the order of toxicity is similar to that observed when fish are immersed in solutions of antimycin. Moreover, the behavior of injected and immersed fish is similar.

Of three species, rainbow trout are the most sensitive to the injected toxicant. Some trout died at 0.01 mg. of antimycin per kg. of body weight, and all died within 24 hours at 0.02 mg./kg. (table 15). Carp are intermediate in sensitivity to injected antimycin, with some dying at 0.14 mg./kg. and all dying at 0.40 mg./kg. or more within 72 hours. In contrast, not less than 20 mg./kg. cause mortalities

among black bullheads, and 30 mg./kg. or more are needed for complete kills within 24 hours.

There were no deaths among the control fish of the three species which were injected with the ethanol-water solvent.

STABILITY OF ANTIMYCIN

The shelf life of acetone-antimycin in stock solutions is good in cool, dark storage. Samples held for 2 years were checked periodically for potency against fingerling rainbow trout. The 96-hour EC_{50} 's and confidence intervals at 12° C. exhibited no greater differences in range than expected when using different lots of the same test species. Similarly, the shelf life of the sand formulation appears to be good if it is kept cool and dry, but the shelf life has not been measured specifically.

Antimycin can be destroyed by exposure to high heat for an adequate period of time. Crystalline antimycin which had been subjected to dry heat at 200° C. for 60 minutes or longer, and then dissolved in acetone and bioassayed was not toxic at 5 or 25 p.p.b. to fingerling rainbow trout within 96 hours (table 16). Crystals which were heated at 200° for only 15 or 30 minutes remained as toxic to fish as the unheated control.

TABLE 15.--Toxicity of intraperitoneal injections of antimycin at 12°C.

Species	Weight (grams)		Injection	Fish alive/dead at--				
	Average	Range		24 hours	48 hours	72 hours	96 hours	120 hours
Rainbow trout.....	33	30-34	0.002	5/0	5/0	5/0	5/0	--
Do.....	33	30-34	0.010	4/1	2/3	2/3	2/3	--
Do.....	33	30-34	0.020	0/5	--	--	--	--
Do.....	33	30-34	Control	5/0	5/0	5/0	5/0	5/0
Carp.....	320	300-330	0.100	5/0	5/0	5/0	5/0	--
Do.....	335	290-390	0.140	--	4/1	2/3	2/3	2/3
Do.....	360	300-430	0.160	--	3/2	3/2	2/3	2/3
Do.....	320	280-360	0.180	--	4/1	2/3	1/4	0/5
Do.....	333	300-370	0.200	5/0	5/0	5/0	4/1	4/1
Do.....	300	280-320	0.400	5/0	2/3	0/5	--	--
Do.....	290	265-310	0.600	5/0	0/5	--	--	--
Do.....	340	310-350	1.000	4/1	0/5	--	--	--
Do.....	325	306-340	Control	5/0	5/0	5/0	5/0	5/0
Black bullhead.....	270	240-300	10.00	4/0	4/0	4/0	4/0	4/0
Do.....	118	100-130	20.00	2/3	2/3	2/3	2/3	2/3
Do.....	121	110-130	25.00	1/4	1/4	1/4	1/4	1/4
Do.....	150	120-170	30.00	0/5	--	--	--	--
Do.....	105	90-140	35.00	0/5	--	--	--	--
Do.....	110	105-120	Control	5/0	5/0	5/0	5/0	5/0

TABLE 16.--Effects of oven-heating at 200° C. on the toxicity of crystalline and sand-formulated antimycin to fingerling rainbow trout at 12°.

[Results presented in numbers of fish alive/dead at selected intervals]

Bioassay time (hours)	Controls	Crystals at 5 p.p.b.				Crystals at 25 p.p.b.				Fintrol-5 at 25 p.p.b.			
		Non-heated	Heated (min.)			Non-heated	Heated (min.)			Non-heated	Heated (min.)		
			15	30	60		30	60	120		30	60	120
0.25.....	30/0	10/0	10/0	10/0	10/0	10/0	10/0	10/0	10/0	10/0	10/0	10/0	10/0
0.50.....	30/0	10/0	10/0	10/0	10/0	10/0	3/7	10/0	10/0	10/0	10/0	10/0	10/0
1.00.....	30/0	10/0	10/0	10/0	10/0	0/10	0/10	10/0	10/0	0/10	8/2	10/0	10/0
2.00.....	30/0	10/0	10/0	10/0	10/0	--	--	10/0	10/0	--	3/9	10/0	10/0
3.00.....	30/0	0/10	0/10	0/10	10/0	--	--	10/0	10/0	--	0/10	10/0	10/0
24.00.....	29/1	--	--	--	10/0	--	--	10/0	10/0	--	--	10/0	10/0
96.00.....	29/1	--	--	--	10/0	--	--	10/0	10/0	--	--	10/0	10/0

DETOXIFICATION WITH POTASSIUM PERMANGANATE

Although antimycin degrades rapidly in water (Derse and Strong, 1963; Walker, Lennon, and Berger, 1964), it may sometimes be desirable to detoxify treated waters. Potassium permanganate was included among candidate detoxifiers because of its activity as a strong oxidizer and the fact that fishery managers are familiar with it.

Crystalline potassium permanganate was dissolved in water and added at concentrations of 5 to 500 p.p.b. to 15-liter bioassay solutions which contained 5 p.p.b. of antimycin at 12° C. Fingerling bluegills were added to the vessels 6 or 24 hours later, and their responses were noted over the following 96 hours. The results reveal that 300 p.p.b. of potassium permanganate detoxifies 5 p.p.b. of antimycin within 6 hours. On the basis of these results, a detailed investigation was

initiated on the detoxification of antimycin by potassium permanganate; this will be reported separately.

DISCUSSION

These studies provide abundant evidence that antimycin has a fine potential as a fishery tool. It is a pure compound which fortunately can be formulated simply and with great integrity. The crystals are easily dissolved in quantities of acetone or ethanol which themselves are not harmful to fish. The formulations in Carbowax coated on fine sand also are precise and uniform. Consequently, fish can be exposed easily to exact quantities of active ingredient and with no influence from the carriers.

Because of these advantages, we are using antimycin as a reference compound in routine bioassays of unknown chemicals. Fish of any species may vary from lot to lot and from season to season in their sensitivity to a chemical. Also, fish within a lot may increase or decrease in sensitivity to a chemical depending on stresses involved in their holding and acclimation at the laboratory. These possible variations in sensitivity are not apparent in tests with compounds of unknown activities. We expose all lots of bioassay fish to antimycin, therefore, as a check on their sensitivity to a toxicant.

The order of toxicity of antimycin to 31 species of fish is summarized in table 17. In static 96-hour bioassays, 1 p.p.b. of the toxicant kills the most susceptible fishes, including trouts, walleye, and yellow perch. Five p.p.b. kill fish of intermediate sensitivity, including carp and green sunfish which are often target coarse fish. Of the 31 species, 27 are eliminated by 10 p.p.b., but up to 25 p.p.b. are needed to kill short-nose gar, bowfin, and channel catfish. White catfish, flathead catfish, and black bullheads are comparatively resistant to antimycin and up to 200 p.p.b. is required for eradication.

TABLE 17.--Order of toxicity of antimycin to 31 fishes, including various life stages at different water temperatures and qualities

1.0 p.p.b.:	5.0 p.p.b.:--Continued
Rainbow trout	Redear sunfish
Brown trout	Pumpkinseed
Brook trout	Green sunfish
Lake trout	Fathead minnow
Walleye	Northern redbelly dace
Yellow perch	Brook stickleback
	Largemouth bass
5.0 p.p.b.:	
White sucker	7.5 - 10.0 p.p.b.:
Smallmouth bass	Goldfish
Freshwater drum	
Black crappie	25 p.p.b.:
Bigmouth buffalo	Shortnose gar
Quillback	Bowfin
Spotted sucker	Channel catfish
Northern pike	
Carp	200 p.p.b.:
Longear sunfish	White catfish
Bluegill	Flathead catfish
	Black bullhead

In addition to the species above, other fish are known to be killed by antimycin. Walker, Lennon, and Berger (1964) listed gizzard shad (Dorosoma cepedianum), stoneroller (Camposoma anomalum), golden shiner (Notemigonus crysoleucas), yellow bullhead (Ictalurus natalis), brown bullhead (Ictalurus nebulosus), white crappie (Pomoxis annularis), and Iowa darter (Etheostoma exile). Howell¹ found that antimycin at 9 p.p.b. is toxic to larvae of the sea lamprey (Petromyzon marinus) within 21 hours. Lowe (1966) determined that the 48-hour EC₅₀ for acetone-antimycin against spot (Leiostomus xanthurus) at 25° C. is 0.23 p.p.b. In his trials of sand-formulated antimycin on juvenile fish, Lowe also found that 10 p.p.b. killed all longnose killifish (Fundulus similis) within 48 hours, and that the 48-hour EC₅₀'s for sea catfish (Galeichthys felis) and sheepshead minnow (Cyprinodon variegatus) at 19° and 23° C. are 10 and 32 p.p.b. respectively. Meyer² reported that grass carp (Ctenopharyngodon idellus) are killed by 3 p.p.b. of antimycin and tilapia (Tilapia mossambica) by 5 p.p.b. within 24 hours in aquaria at room temperature.

¹ Letter from John Howell, Supervisory Fishery Biologist, Hammond Bay Biological Station, Bureau of Commercial Fisheries, Millersburg, Mich., 1966.

² Letter from Dr. Fred P. Meyer, Chief, Fish Farming Experimental Station, Bureau of Sport Fisheries and Wildlife, Stuttgart, Ark., 1966.

Antimycin has another advantage as a fish control agent: fish are not repelled or excited by its presence in water. This, coupled with the order of toxicity and effectiveness on various life stages, suggests that the toxicant has possibilities for partial or selective control of fish. The lethal effects on fish eggs, for example, indicate that it might be employed effectively in spawning areas for partial control of unwanted species. Or it might be useful for control of trash fish in catfish production ponds. There are also possibilities worth investigating that the sand formulations, Fintrol-5 and Fintrol-15, might be used in littoral zones or epilimnions of thermally stratified lakes to remove stunted or unwanted fish.

Certain facts disclosed by the studies point to a possible usefulness of antimycin in reclaiming streams. They include the lack of color, odor, or repellency in the toxicant, the relatively brief exposures needed to kill fish, the relatively rapid rate of degradation, and the susceptibility of the antibiotic to detoxification with potassium permanganate.

Also, from the point of view of fishery management, the toxicant is effective in a variety of water qualities. High pH contributes to more rapid degradation of antimycin, but this might be an advantage in certain situations so long as the concentrations applied persist through an effective contact time. Temperature has less effect on the activity of the toxicant than pH, but somewhat greater concentrations may be required in very cold waters and under ice than in waters of moderate temperature. Water hardness has less influence on antimycin than pH or temperature, but toxicity is slightly greater in soft water than in hard water.

A mineral turbidity may retard or reduce the toxicity of antimycin to fish, depending on the amount of material in suspension and whether it remains in suspension or settles out. Similarly, Ferguson et al. (1966) reported that muds reduce the bioactivity of chlorinated hydrocarbon insecticides on fish.

As an outgrowth of these laboratory trials, the development of antimycin as a fishery tool has begun to include comprehensive tests of its toxicity to birds and mammals (Vezina, 1967), and experimental applications against fish in lakes and streams (Lennon, 1966; Berger, 1966). Lennon, Berger, and Gilderhus (1967) adapted seed spreaders for distributing sand-formulated antimycin in field operations. Powers and Bowes (1967) used antimycin to control predaceous fish in a grebe refuge in Guatemala. Hogan (1967) and Burress and Luhning (1969) investigated the utility of antimycin in channel catfish production. Walker (1967) has further defined the amounts of potassium permanganate needed to detoxify various concentrations of antimycin in water.

CONCLUSIONS

1. Antimycin in water or injected intraperitoneally is toxic to fish. Various life stages of some species of fish were included in tests, and the antibiotic was toxic to all, egg through adult.
2. Solutions of antimycin crystals in acetone or ethanol or dry preparations in Carbowax on sand are stable in storage, irreversibly toxic to fish in waters of various qualities and temperatures, and subject to detoxification by potassium permanganate.
3. Natural degradation occurs sooner in hard, alkaline waters than in soft, acid waters. The degradation products are not toxic to fish. The exposure of the antibiotic to dry heat at 200° C. for short periods of time reduces or destroys its toxicity to fish.
4. The purity of the compound, the integrity of its formulations, and its lack of repellency to fish are characteristics which enhance its use for general, partial, or selective control of unwanted fish.

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INVESTIGATIONS IN FISH CONTROL

**27. Field Trials of Antimycin A
as a Fish Toxicant**

By Philip A. Gilderhus, Fishery Biologist,
Bernard L. Berger, Chemist, and
Robert E. Lennon, Fishery Biologist



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FIELD TRIALS OF ANTIMYCIN A AS A FISH TOXICANT

By Philip A. Gilderhus, Bernard L. Berger, and Robert E. Lennon
Bureau of Sport Fisheries and Wildlife
Fish Control Laboratory, La Crosse, Wisconsin

ABSTRACT.--Antimycin A was subjected to field trials as a fish toxicant in 20 ponds and lakes and 5 streams in the East, Midwest, and West of the United States. The formulations of toxicant included three on sand grains which are designed to release antimycin uniformly within certain depths, and one formulation in a liquid. Ten parts per billion or less of the toxicant were effective against most of the 54 species of fish we encountered, including carp, suckers, and green sunfish. Differences in sensitivity among fish suggest possible use of antimycin as a selective toxicant. The efficacy of the toxicant is influenced by pH and water temperature, with slightly higher concentrations necessary at high pH or in cold water. Antimycin does not repel fish, and its toxic action on fish appears to be irreversible. It degrades rapidly, usually within a week. Fish-killing concentrations have little or no effect on other aquatic animals.

The development and registration of a fish toxicant require that the candidate chemical be tested thoroughly in the laboratory and in the field to evaluate its toxicity to different forms of invertebrate and vertebrate life, its efficacy on fish, and its residues in water and fish. The trials in the field must involve at least three ecologically different sites, and target and other organisms typical of those sites. Also, they must be organized, executed, and reported as controlled experiments, and the data from them are reviewed in detail by regulatory agencies at Federal and State levels before the toxicant may be registered for use. Notably, the regulatory authorities warn against submitting the once-common testimonial type of reports on field trials.

The field trials of antimycin A followed intensive testing in the laboratory which demonstrated its potential as a toxicant specific to fish (Walker et al., 1964; Vezina, 1967; Herr et al., 1967; and Berger et al., 1969). The sites were chosen and the trials conducted with the requirements for registration in mind. Thus, waters in the East, Midwest,

and West of the United States were included. Soon after each of the early trials, a report was submitted to the Pesticide Regulation Division of the U.S. Department of Agriculture for review and for suggestions on points to consider in subsequent trials.

The objectives of the field trials were:

1. to evaluate the efficacy of antimycin A in several formulations against as many species of fish as possible in cold and warm waters, acid and alkaline waters, and clear and turbid waters,
2. to determine concentrations of antimycin which are effective against such prime target fish as carp, suckers, and green sunfish,
3. to observe any effects of the toxicant on other aquatic animals,
4. to detect whether or not the toxicant repels fish,

5. to find out if the toxic action in fish is irreversible,
6. and to observe the rates of natural and induced degradation of antimycin in water.

The early trials were conducted on very small ponds for two reasons. First, the tests were easier to control and evaluate than in larger waters. Second, the quantities of formulated antimycin were limited because pharmacologists of the producing company were then in the midst of developing and perfecting the Fintrol formulations.

Concurrently, field trials by other investigators bolstered the development of antimycin as a fish toxicant (Loeb, 1964; Lennon, 1966; Lennon et al., 1967; Powers and Bowes, 1967; Powers and Schneberger, 1967; Radonski, 1967; Burress, 1968; and Burress and Luhning, 1969a and 1969b).

The successful accomplishment of field trials was made possible only through the generous cooperation of many people. We are indebted especially to fishery personnel of State conservation departments who provided experimental sites, stocked fish, and manpower; to fishery and wildlife personnel in Bureau hatcheries, refuges, and laboratories who also provided trial sites, fish, and aid; to Wisconsin Alumni Research Foundation, owners of antimycin, for advice and aid; to Ayerst Laboratories, producers of antimycin, for supplying the pure and formulated toxicant at no charge; and to personnel of the Pesticide Regulation Division, U.S. Department of Agriculture, for counsel on the scope and depth of experimental data needed for registration of antimycin as a fishery tool.

MATERIALS AND METHODS

Antimycin A

The early development of antimycin A as a fish toxicant included much consideration and investigation of formulations to meet the requirements of fish managers. A liquid formulation was needed for application in streams and in the very shallow waters of ponds and

marshes. Accordingly, an experimental Fintrol-Liquid was prepared, consisting of crystalline antimycin dissolved in acetone or ethanol with a small amount of surfactant added.

We recognized the long-standing problem that fish managers have experienced in getting adequate distribution of liquid or powdered toxicants into the deeper waters of ponds and lakes, especially when thermal stratification exists. We sought granular formulations, heavier than water, which would release antimycin within selected depths. Thus, the cumbersome and often imperfect job of pumping a toxicant into the depths of a lake would be avoided. As a result of cooperative efforts among scientists of Ayerst Laboratories, Wisconsin Alumni Research Foundation, and the Bureau of Sport Fisheries and Wildlife, experimental formulations of antimycin on sand were developed.

The sand formulations consist of antimycin incorporated in Carbowax^(R) and coated on sand in such a way that the toxicant is released into the water within certain depths as the sand grains sink toward the bottom. Because we expected that most of the early applications of antimycin would be in production ponds, farm ponds, and small lakes, we chose formulations on sand which would release the toxicant evenly within the first 5, 15, and 30 feet of depth. They are called Fintrol-5, Fintrol-15, and Fintrol-30, respectively. Of them, only Fintrol-5 is registered and on the market. Formulations for release of toxicant over greater depths or for delayed release on the bottom are possible and may be developed later.

All of the formulated antimycin used in the field trials was prepared and furnished by Ayerst Laboratories, New York, N.Y.

The liquid formulation of antimycin was applied to streams and shallows of ponds by means of spray apparatus or metering pumps. In most cases, a strong solution of Fintrol-Liquid in water was prepared on site and promptly dispensed. Metering pumps of the type described by Anderson (1962) gave the best performance.

The formulations of antimycin on sand were broadcast by hand, by hand-operated seed spreader, or by powered seed spreaders mounted on boats (Lennon et al., 1967). An attempt was made on each job to achieve as rapid and as even distribution as possible.

Degradation or Detoxification of Antimycin

The degradation of the toxicant in ponds was determined by field bioassays. Fingerling rainbow trout or bluegills were placed in live cages and submerged at selected sites and depths in the ponds at least 24 hours prior to the application of antimycin. Each live cage contained at least 10 specimens of a species and size of fish.

Following application of the toxicant, the fish in live cages were checked frequently during the first 36 to 48 hours to detect the rate and amount of mortality. As soon as all specimens of a species in a live cage were dead, a replacement lot was provided. The survival of all specimens in a live cage for at least 48 hours was indicative that the toxicant had degraded below the lethal concentration or had flowed from the vicinity.

Potassium permanganate was used during two field trials to detoxify the antimycin in effluent waters. Solutions or crystals of

KMnO₄ were applied to obtain concentrations of 1 to 2 p.p.m. in the water.

Sites of Field Trials

The 13 sites selected for field trials of antimycin included 20 ponds or lakes and 5 streams (tables 1 and 2). The first trials were conducted on Bureau property and involved 14 ponds which are not subject to public fishing. These selections were made not only to obtain good tests of the toxicant, but also as a precaution against undue risks to the public until we knew more about the performance of antimycin in the field.

The ponds and lakes ranged in area from 0.25 to 63 surface acres and in volume from 0.45 to 787 acre-feet (table 2). The streams were small and ranged in volume of flow from 2 to 20 c.f.s. The ranges in water constituents were: pH, 7.0 to 8.8; total hardness, 10 to 350 p.p.m.; and total alkalinity, 13 to 350 p.p.m. (tables 2 and 3). Water temperatures ranged from 5° to 20° C., and all temperatures in this report are expressed in Celsius.

Fifty-four species of freshwater fish were represented in the trials, and they included such problem species as carp, white suckers, and green sunfish (table 4). In the early trials in small ponds, known numbers and sizes of fish were stocked to facilitate evaluations on the performance of the toxicant.

TABLE 1.--Sites of field trials with antimycin A

Town and state	Site	Owned or managed by
<u>Ponds and lakes</u>		
1. Berlin, N.H.....	Berlin NFH ¹	Bureau of Sport Fisheries and Wildlife
2. Cape Vincent, N.Y..	Cape Vincent NFH	Bureau of Sport Fisheries and Wildlife
3. Stuttgart, Ark.....	Warmwater Fish Cultural Laboratory	Bureau of Sport Fisheries and Wildlife
4. Valentine, Neb.....	Valentine NWR ²	Bureau of Sport Fisheries and Wildlife
5. Saratoga, Wyo.....	Saratoga NFH	Bureau of Sport Fisheries and Wildlife
6. West Salem, Wis....	Veterans Memorial Park	La Crosse County, Wisconsin
7. Madison, Wis.....	Lake Katrine	State of Wisconsin
8. Oxford, Wis.....	Parker Lake	State of Wisconsin
<u>Streams</u>		
9. Viroqua, Wis.....	Sidie Hollow Creek	State of Wisconsin
10. Plymouth, Wis.....	Mullet River	State of Wisconsin
11. Cataract, Wis.....	Rathbone Creek	State of Wisconsin
12. Westfield, Wis.....	Westfield Creek	State of Wisconsin
13. Marshall, Wis.....	Waterloo Creek	State of Wisconsin

¹ National Fish Hatchery

² National Wildlife Refuge

TABLE 2.--Physical and chemical characteristics of ponds and lakes

Site	Date	Pond or lake	Surface area (acres)	Volume (acre-feet)	Water temp. (°C.)	Chemical characteristics				
						DO (p.p.m.)	CO ₂ (p.p.m.)	pH	Total hardness (p.p.m.)	Total alkalinity (p.p.m.)
1. Berlin NFH.....	9/64	P1	--	0.45	12	9.6	0.1	7.0	10.0	14.2
		P2	--	1.16	13	9.4	0.1	7.2	10.0	12.8
		P3	--	0.55	12	9.0	0.1	7.2	10.0	14.5
2. Cape Vincent NFH	10/64	P1	1.00	2.93	12	8.9	2.5	8.1	153.0	110.5
		P2	1.00	2.55	12	8.7	3.0	7.9	159.0	118.0
		P3	1.00	2.74	12	8.0	3.2	8.1	155.0	118.0
3. Warmwater Fish Cultural Laboratory.	3/65	P1	0.25	0.58	17	9.6	1.0	8.0	59.0	81.0
		P2	0.25	0.58	17	10.4	1.0	8.1	58.0	78.0
		P3	0.25	0.58	17	9.6	1.0	8.2	55.0	72.0
		P4	0.25	0.58	17	10.4	1.0	8.2	58.0	77.0
		P5	0.25	0.58	17	9.6	1.0	8.1	60.0	79.0
		P6	0.25	0.58	17	10.4	1.0	8.2	52.0	70.0
	12/65	I1	19.40	42.68	12	10.8	--	8.6	112.0	110.0
		I2	0.25	0.58	12	7.4	--	7.9	120.0	148.0
4. Valentine NWR...	5/65	P1	1.80	3.24	19	8.3	0.0	8.4	107.0	155.0
		P2	0.50	1.95	20	--	0.9	7.9	142.0	258.0
5. Saragoga NFH....	10/65	--	1.79	5.84	9	--	--	7.7	237.0 ¹	184.0
6. Veterans Memorial Park.	11/65	--	4.78	21.51	13	--	--	7.8	223.0	188.0
7. Lake Katrine....	5/66	--	2.70 ²	10.01	17	--	--	6.8-8.5	32.0	25.0
	10/66	--	30.00	118.00	18	--	--	8.2	--	35.0
8. Parker Lake.....	10/66	--	63.00	787.00	9	--	--	8.2	197.0	--

¹ Total dissolved solids² Partial treatment (small bay)

TABLE 3.--Physical and chemical characteristics of streams

Site	Date	Flow (c.f.s.)	Length of treated section (mi.)	Width (ft.)	Water temp. (°C.)	Chemical characteristics	
						pH	Total hardness (p.p.m.)
9. Sidie Hollow Creek.	8/65	1.76	3.5	4	10 -18	8.3	240
10. Mullet River.....	9/66	10.0	2.5	18	20 -22	8.4	210
11. Rathbone Creek.....	11/66	10.7	6.0	9	5	7.5	24
12. Westfield Creek....	6/67	21.7	3.0	20	20	8.8	170
13. Waterloo Creek.....	7/67	4.3	1.0	30	25	8.6	350

TABLE 4.--Fish exposed to antimycin in field trials

Common name	Scientific name	Sites where found (by number)
American brook lamprey.....	<u>Lampetra lamottei</u>	11, 12
Paddlefish.....	<u>Polyodon spathula</u>	3
Bowfin.....	<u>Amia calva</u>	2
Gizzard shad.....	<u>Dorosoma cepedianum</u>	3
Rainbow trout.....	<u>Salmo gairdneri</u>	1, 2, 3, 6, 8, 9, 12
Atlantic salmon.....	<u>Salmo salar</u>	1
Brown trout.....	<u>Salmo trutta</u>	2, 6, 9, 10
Brook trout.....	<u>Salvelinus fontinalis</u>	1, 2, 5, 11
Central mudminnow.....	<u>Umbra limi</u>	6, 10, 11
Chain pickerel.....	<u>Esox niger</u>	1
Northern pike.....	<u>Esox lucius</u>	2, 4, 6, 10, 13
Stoneroller.....	<u>Camptostoma anomalum</u>	9
Goldfish.....	<u>Carassius auratus</u>	3
Carp.....	<u>Cyprinus carpio</u>	2, 3, 4, 6, 7, 8, 10, 12, 13
Golden shiner.....	<u>Notemigonus crysoleucas</u>	1, 2, 3, 6
Common shiner.....	<u>Notropis cornutus</u>	1
Bluntnose minnow.....	<u>Pimephales notatus</u>	12
Fathead minnow.....	<u>Pimephales promelas</u>	4, 5, 6, 7, 12
Blacknose dace.....	<u>Rhinichthys atratulus</u>	1, 9, 12
Longnose dace.....	<u>Rhinichthys cataractae</u>	9
Creek chub.....	<u>Semotilus atromaculatus</u>	1, 5, 9, 10, 12
Fallfish.....	<u>Semotilus corporalis</u>	1
White sucker.....	<u>Catostomus commersoni</u>	1, 2, 6, 9, 10, 11, 12, 13
Northern hog sucker.....	<u>Hypentelium nigricans</u>	9
Smallmouth buffalo.....	<u>Ictiobus bubalus</u>	3
Bigmouth buffalo.....	<u>Ictiobus cyprinellus</u>	3
Buffalo hybrid.....	<u>Ictiobus sp.</u>	3
White catfish.....	<u>Ictalurus catus</u>	3
Blue catfish.....	<u>Ictalurus furcatus</u>	3
Black bullhead.....	<u>Ictalurus melas</u>	3, 4, 6, 7
Yellow bullhead.....	<u>Ictalurus natalis</u>	3
Brown bullhead.....	<u>Ictalurus nebulosus</u>	1, 2
Channel catfish.....	<u>Ictalurus punctatus</u>	3, 6
Tadpole madtom.....	<u>Noturus gyrinus</u>	3
Flathead catfish.....	<u>Pylodictus olivaris</u>	3
Mosquitofish.....	<u>Gambusia affinis</u>	3
Brook stickleback.....	<u>Eucalia inconstans</u>	9, 11, 12
White bass.....	<u>Roccus chrysops</u>	2
Rock bass.....	<u>Ambloplites rupestris</u>	2, 10
Warmouth.....	<u>Chaenobryttus gulosus</u>	3
Green sunfish.....	<u>Lepomis cyanellus</u>	3, 4, 6, 10
Pumpkinseed.....	<u>Lepomis gibbosus</u>	1, 2, 6, 8, 12
Orangespotted sunfish.....	<u>Lepomis humilis</u>	3
Bluegill.....	<u>Lepomis macrochirus</u>	1, 2, 3, 4, 6, 8, 10, 12
Smallmouth bass.....	<u>Micropterus dolomieu</u>	1, 2
Largemouth bass.....	<u>Micropterus salmoides</u>	1, 2, 3, 4, 10, 12
White crappie.....	<u>Pomoxis annularis</u>	6
Black crappie.....	<u>Pomoxis nigromaculatus</u>	2, 3, 4, 10
Iowa darter.....	<u>Etheostoma exile</u>	5
Fantail darter.....	<u>Etheostoma flabellare</u>	12
Johnny darter.....	<u>Etheostoma nigrum</u>	9, 10, 11, 12
Yellow perch.....	<u>Perca flavescens</u>	1, 2, 4, 8
Walleye.....	<u>Stizostedion vitreum vitreum</u>	2
Freshwater drum.....	<u>Aplodinotus grunniens</u>	2
Mottled sculpin.....	<u>Cottus bairdi</u>	9, 12

Pre- and Post-treatment Surveys

STANDING WATER

The ponds and lakes were mapped carefully, including depths and contours. Their volumes in acre-feet were calculated from measurements of area and depth. Analyses of water chemistry were made on site and correlated with any historical data available. The species, abundance, and sizes of fish were assessed before the trials from stocking records, or by netting or electrofishing. The post-treatment presence of fish was detected by netting, electrofishing, or by draining the ponds.

The populations of bottom fauna and plankton were sampled by standard techniques before and after applications of antimycin, and attempts were made to correlate changes in composition or abundance with causative factors.

FLOWING WATER

The streams were mapped and sounded, and the volumes and velocities were measured with a current meter. Measurements of stretchout and dilution were accomplished in soft water by the salt-resistivity technique (Lennon, 1959; Lennon and Parker, 1959) and in hard water by use of the rhodamine-fluorometer technique (Buchanan, 1964). The losses in concentration of salt or rhodamine dye between selected stations on a stream were assumed to equal the losses of a toxicant in the same flow and distance. On the basis of these data, the sites of fortifying stations were located and the amounts of toxicant to be added at each station were determined.

Analyses of the water were made routinely by standard techniques. The populations of fish were sampled before and after applications of antimycin by electrofishing.

RESULTS

Trials in Ponds and Lakes

BERLIN, NEW HAMPSHIRE

Three, small earth ponds were selected at the Berlin National Fish Hatchery as typical of cold, soft, trout water in northern New England (tables 2, 5, and 6). Sixteen species of wild and hatchery-reared fish were stocked in known numbers at least 1 week prior to the experimental reclamation.

We chose a wide range of concentrations of antimycin because this was the first trial in soft water. One pond was treated with 0.13 p.p.b., another with 1.22 p.p.b., and the third with 12.00 p.p.b. It was also the first field trial of the sand formulation, Fintrol-5.

Nine of the 12 species exposed to 0.13 p.p.b. suffered mortalities. Only common shiners had a complete kill; the other species had partial mortalities ranging from 18 to 75 percent. The fish in this pond, however, received only a 24-hour exposure to the toxicant. There was a large loss of water from the pond because of seepage, and fresh water had to be added each 24 hours. The fish unaffected by the relatively brief exposure to the low concentration were chain pickerel, brown bullheads, and bluegills.

The toxicant at 1.22 p.p.b. caused complete kills of 12 species within 72 hours, and it degraded in 120 hours. A few bluegills and smallmouth bass and all brown bullheads survived.

The 12 p.p.b. of antimycin killed the most sensitive species within 10 hours and all fish except brown bullheads within 48 hours. More than 2 weeks were required for this concentration of toxicant to degrade below fish-killing levels.

TABLE 5.--Applications of antimycin and rates of degradation in field trials

Site	Pond or lake	Formulation (percent active)	Total formulation	Antimycin (g.)	Conc. of antimycin in water (p.p.b.)	Degradation time (hrs.)
1. Berlin NFH.....	P1	Fintrol-5, 0.19	38 g.	0.07	0.13	24
	P2	Fintrol-5, 0.19	940 g.	0.76	1.22	120
	P3	Fintrol-5, 0.88	1,000 g.	8.22	12.00	>330
2. Cape Vincent NFH...	P1	Fintrol-5, 0.10	3,790 g.	3.70	1.02	96
	P2	Fintrol-5, 0.29	3,395 g.	9.85	3.12	96
	P3	Fintrol-5, 0.96	3,675 g.	35.40	10.40	96
3. Warmwater Fish Cultural Laboratory March 1965	P1	Fintrol-5, 1.0	360 g.	3.60	5.00	168
	P2	Fintrol-5, 1.0	360 g.	3.60	5.00	168
	P3	Fintrol-5, 1.0	540 g.	5.40	7.50	168
	P4	Fintrol-5, 1.0	540 g.	5.40	7.50	168
	P5	Fintrol-5, 1.0	720 g.	7.20	10.00	168
	P6	Fintrol-5, 1.0	720 g.	7.20	10.00	168
December 1965....	L1	Fintrol-5, 1.0	53 kg.	526.64	10.00	120
	L2	Fintrol-5, 1.0	400 g.	4.00	10.00	120
4. Valentine NWR.....	P1	Fintrol-5, 1.0	4,018 g.	40.18	10.00	24
	P2	Fintrol-5, 1.0	2,480 g.	24.80	10.00	120
5. Saratoga NFH.....		Fintrol-5, 1.0	17 kg.	17.20	10.00	--
6. Veterans Memorial Park.		Fintrol-5, 1.0	22 kg.	220.00	10.00	144
7. Lake Katrine May 1966..... Oct. 1966.....		Fintrol-5, 1.0	5,000 g.	50.00	4.00	96
		Fintrol-5, 1.0	431 kg.	4,313.00	7.50	120
8. Parker Lake.....		Fintrol-5, 1.0	288 kg.	2,883.00	10.00	--
		Fintrol-15, 1.5	175 kg.	2,647.00	--	--
		Fintrol-30, 3.2	159 kg.	5,130.00	--	168
		Liquid	--	100.00	--	--
9. Sidie Hollow Creek.		Liquid	--	--	15 for 5 hours	--
10. Mullet River..... (Plymouth Pond)		Liquid, 0.37-0.52	43 l.	206.50	7.5 for 8 hours	--
11. Rathbone Creek..... (Cataract Pond)		Liquid, 10.0	1,540 ml.	154.00	7.5 for 8 hours	--
		Fintrol-5, 1.0	11.6 kg.	115.70	7.5	--
12. Westfield Creek....		Liquid, 10.0	6,250 ml.	625.00	10 for 10 hours	--
13. Waterloo Creek.....		Liquid, 10.0	1,825 ml.	182.00	10 for 5 hours	--

TABLE 6.--Effects of antimycin on fish at Berlin NFH

Species	Size (inches)	Number per pond	Percent killed at		
			0.13 p.p.b.	1.22 p.p.b.	12.0 p.p.b.
Rainbow trout.....	5.3-5.9	115-116	18	100	100
Atlantic salmon.....	3.4-3.7	21-22	--	100	100
Brook trout.....	4.0-4.6	111-112	46	100	100
Chain pickerel.....	10.0-12.0	11-12	0	100	100
Golden shiner.....	7.7-8.1	4	--	--	100
Common shiner.....	4.7-5.0	3-4	100	100	--
Blacknose dace.....	2.0	13-14	--	100	100
Creek chub.....	3.8-4.0	8	--	--	--
Fallfish.....	4.6-4.8	8-9	50	100	100
White sucker.....	9.8-10.6	16	31	100	100
Brown bullhead.....	10.0-10.5	9-10	0	0	0
Pumpkinseed.....	5.5-6.3	12-14	50	100	100
Bluegill.....	7.5-7.9	9	0	44	100
Smallmouth bass.....	4.5-4.8	23-28	38	86	100
Largemouth bass.....	3.6-6.0	8-11	75	100	100
Yellow perch.....	7.0-8.7	12	33	100	100

The results at Berlin NFH were important to subsequent field trials and to further development of the toxicant. Although the 0.13 p.p.b. of antimycin was too small a concentration for a large kill of fish, the no-kill and partial-kill of certain species confirmed laboratory findings that some species are more sensitive than others, and that antimycin has potentials for selective control of sensitive fish. The concentration of 1.22 p.p.b. was also too small for a complete kill of scale fish. On the other hand, the concentration of 12 p.p.b. was too high. We concluded that the optimum concentration for scale fish in the cold, soft water might have been 4 or 5 p.p.b.

The results also confirmed laboratory observations that bullheads are very tolerant of concentrations of antimycin which kill most scale fish. This could be an advantage in some situations, and a disadvantage in others. We concluded, however, that the presence or absence of bullheads could be ignored in most of our field trials because the amounts of toxicant used would be too small to affect them.

This first test of antimycin formulated on sand, Fintrol-5, demonstrated that the preparation is easy and clean to handle and distribute, and the results supported a decision to continue development of sand formulations. We did recommend that a coarser grade of

sand be used as the carrier because the fine sand could float on surface film of water or could be blown from the path or distribution by the wind. The manufacturer then changed from 60-mesh sand to 40-mesh.

The pond waters were clear and colorless and afforded good observation of the fish during and after the application of antimycin. The formulation contributed no color or odor to the water, and the fish were not repelled by it. Moreover, it killed the fish slowly and unspectacularly.

CAPE VINCENT, NEW YORK

Three 1-acre ponds at the Cape Vincent NFH were stocked with 20 species of fish and treated with the sand formulation of antimycin. The water was harder and more fertile than at Berlin NFH, and the ponds contained substantial amounts of rooted, aquatic vegetation.

We narrowed the range of test concentrations to 1.02, 3.12, and 10.4 p.p.b. of antimycin (table 7). The lowest concentration killed all trouts, certain minnows, white suckers, and yellow perch. Seven species had partial mortalities ranging from 17 to 97 percent, and seven species had no mortalities. Only bowfin and brown bullheads and some of the pumpkinseeds, smallmouth bass, largemouth bass, and black crappies survived

TABLE 7.--Effects of antimycin on fish at Cape Vincent NFH

Species	Size (inches)	Number per pond	Percent killed at		
			1.02 p.p.b.	3.12 p.p.b.	10.4 p.p.b.
Bowfin.....	16.2-18.8	1	0	0	0
Rainbow trout.....	7.7-9.0	5-10	100	100	100
Brown trout.....	9.0-10.3	5-6	100	100	100
Brook trout.....	9.9-10.7	5-6	100	100	100
Northern pike.....	14.5-17.1	2-3	0	100	100
Carp, small.....	2.3-2.5	100	94	100	100
Carp, large.....	16.8-17.4	1	0	100	--
Minnows.....	2.4-3.0	70-80	100	100	100
Golden shiner.....	2.9-3.1	29	97	100	100
White sucker.....	17.1-19.2	2-8	100	100	100
Brown bullhead.....	4.2-5.3	29-30	0	0	0
White bass.....	15.4	0-1	0	--	--
Rock bass.....	7.0-8.0	7-8	17	100	100
Pumpkinseed.....	4.5-6.2	10-22	45	88	100
Bluegill.....	7.6-8.2	9-30	88	100	100
Smallmouth bass.....	11.5	0-1	0	63	100
Largemouth bass.....	12.0-14.0	19	0	63	100
Black crappie.....	6.8-8.0	10-12	70	67	100
Yellow perch.....	7.2-8.0	9	100	100	100
Walleye.....	12.1-16.5	4-5	50	100	100
Freshwater drum.....	16.0-18.0	1-2	0	100	100

exposure to 3.12 p.p.b. Notably, the species eliminated by this concentration included juvenile and adult carp, minnows, and white suckers.

Exposure to 10.4 p.p.b. of antimycin killed 17 of the 19 species, leaving only bowfin and brown bullheads. Comparing the results obtained with 3.12 and 10.4 p.p.b., we concluded that the latter concentration was too high for most of the species involved in the trials.

The sand formulation worked well in the presence of aquatic plants. The sand sank through the submerged weeds, and it sifted nicely through some dense patches of emergent vegetation with no sticking to leaves and stalks. Its advantages over liquid toxicants in weedy water were very apparent.

Among the abundant invertebrates in the ponds, the fresh-water shrimp and aquatic insects were unaffected by the antimycin. Rotifers, copepods, and cladocerans, however, were reduced in numbers in ponds treated with 3.12 and 10.4 p.p.b., but there was question whether the declines of the autumnal pulses were natural or caused wholly by the toxicant.

STUTTQUART, ARKANSAS

Two series of trials were conducted in 1965 at the Bureau's Warmwater Fish Cultural Laboratory. The first, in March, included three pairs of one-quarter acre ponds which were stocked heavily with fish. We narrowed the range of concentrations further, and

treated the ponds with 5.0, 7.5, and 10.0 p.p.b. of antimycin in sand formulation. The lowest concentration eliminated 12 of the 14 species, including such important target fish as goldfish, carp, and green sunfish (table 8). There was a partial kill of channel catfish and warmouth. The concentrations of 7.5 and 10.0 p.p.b. removed all species but channel catfish which suffered 3-percent mortalities at each concentration. An examination of the dead catfish disclosed that they were hosts to heavy infestations of *Ichthyophthirius*.

The second series of trials took place in December in a 20-acre reservoir and a 0.25-acre pond. The fish in the reservoir were well-established populations, some of which were seined and stocked in the small pond. The waters were turbid, and colder and harder than during the March trials. Consequently, we elected to try a concentration of 10 p.p.b. of antimycin in each body of water.

The experimental conditions were heightened because the reservoir had been drawn down to an average depth of about 2 feet, and the bottom was very soft mud. The small pond, on the other hand, ranges from 3 to 4 feet in depth, and the bottom is relatively firm. A comparison of results with Fintrol-5 would indicate whether there was significant loss of antimycin in the reservoir because the sand particles sank into the soft muck before releasing all of the toxicant.

Arrangements also were made to determine the minimum duration of exposure necessary to kill a species of fish. Live cages were

TABLE 8.--Effects of antimycin on fish at the Warmwater Fish Cultural Laboratory, March 1965

Species	Size (inches)	Number per pond	Percent killed at		
			5.0 p.p.b.	7.5 p.p.b.	10.0 p.p.b.
Rainbow trout.....	4.9-9.1	159-176	100	100	100
Goldfish, small.....	0.8-1.4	237-321	100	100	100
Goldfish, large.....	3.0-8.5	643-728	100	100	100
Carp (Israeli), small....	3.5-6.0	530-688	100	100	100
Carp (Israeli), large....	23.0-26.0	2-6	100	100	100
Golden shiner.....	2.8-4.5	302-511	100	100	100
Smallmouth buffalo.....	5.5-13.0	32-72	100	100	100
Bigmouth buffalo.....	12.0-20.0	76-99	100	100	100
Channel catfish.....	12.0-18.0	43-99	2	3	3
Warmouth.....	3.5-5.5	14-26	85	100	100
Green sunfish.....	2.5-7.0	171-273	100	100	100
Orangespotted sunfish....	2.5-3.0	39-59	100	100	100
Bluegill.....	4.0-6.5	46-56	100	100	100
Largemouth bass.....	10.0-12.0	28-40	100	100	100

placed in the reservoir and each contained 10 adult bigmouth buffalo. After a 2-, 3-, 4-, 5- or 6-hour exposure to antimycin, the fish were transferred to an untreated pond. Subsequent observations revealed that the fish exposed to antimycin for 2 hours survived. Those fish exposed to the toxicant for 3 or more hours died.

Among the 19 species present in the reservoir, gizzard shad were the most sensitive to the toxicant, and all died in about 24 hours (table 9). The carp, buffalo, and most other species died within 72 hours. Survivors included paddlefish, catfishes, mosquitofish, and a few warmouth.

The toxicant produced a much quicker kill of fish in the 0.25-acre pond, with heavy mortalities occurring within 24 hours. All species except paddlefish and catfishes were dead at 48 hours. All of the paddlefish, however, succumbed in about 72 hours in contrast with no kill of that species in the reservoir.

The trial demonstrated that antimycin works well in hard, cold, turbid water against scalefish without harm to 7 species of catfish. Among the scalefish, mosquitofish and warmouth were relatively tolerant to the toxicant.

The more rapid and complete kill of fish in the deeper, 0.25-acre pond was attributed to

a better release of toxicant than occurred in the reservoir. We believe that the sand particles sank into the soft bottom of the reservoir before releasing all of the antimycin. Thus, there is risk of an incomplete kill if the 5-foot-release formulation is used in very shallow water over soft bottom.

VALENTINE, NEBRASKA

The first tests of antimycin in natural ponds occurred at Valentine NWR. Pond 1 has an average depth of only 1.8 feet and a relatively soft bottom. The target fish were wild, yearling carp and green sunfish. In addition, some northern pike and largemouth bass from a nearby lake were stocked prior to the trial (table 10). Pond 2 is an old dugout pond with an average depth of 4.2 feet, and at the time, it had overflowed its banks onto adjacent pasture. This pond receives heavy use by range cattle, and it is highly polluted by manure in the water and along the shore. It contained seven species of stocked fish, including large carp.

Both ponds were treated with the Fintrol-5 formulation although the average depths were less than 5 feet. A concentration of 10 p.p.b. was selected because of the relatively high total alkalinity.

Five species of fish were eliminated from Pond 1 within 48 hours. They included the

TABLE 9.--Effects of antimycin on fish at the Warmwater Fish Cultural Laboratory, December 1965

Species	Size (inches)	Reservoir (10 p.p.b.)		Pond (10 p.p.b.)	
		Abundance	Percent killed	Abundance	Percent killed
Paddlefish.....	43.0-57.0	scarce	0	scarce	100
Gizzard shad.....	5.3-6.6	abundant	100	scarce	100
Goldfish.....	5.0-8.0	scarce	100	--	--
Carp.....	10.4-13.8	common	100	abundant	100
Bigmouth buffalo.....	7.2-19.6	common	100	abundant	100
White catfish.....	unknown	scarce	0	scarce	0
Blue catfish.....	unknown	scarce	0	--	--
Black bullhead.....	unknown	common	0	scarce	0
Yellow bullhead.....	unknown	scarce	0	scarce	0
Channel catfish.....	unknown	scarce	0	--	--
Tadpole madtom.....	unknown	common	0	--	--
Flathead catfish.....	unknown	scarce	0	--	--
Mosquitofish.....	1.6-2.8	common	99	--	--
Warmouth.....	3.0-6.5	abundant	99	--	--
Green sunfish.....	3.1-7.6	common	100	--	--
Orangespotted sunfish...	1.7-3.5	common	100	--	--
Bluegill.....	2.5-4.2	scarce	100	--	--
Largemouth bass.....	5.3-16.0	common	100	--	--
Black crappie.....	3.2-9.7	scarce	100	--	--

TABLE 10.--Effects of antimycin on fish at Valentine NWR

Species	Size (inches)	Pond 1 (10 p.p.b.)		Pond 2 (10 p.p.b.)	
		Number present	Percent killed	Number present	Percent killed
Northern pike.....	14.0-24.0	6	100	12	33
Carp.....	10.0-26.0	190	100	1,140	100
Fathead minnow.....	1.8-2.9	0	--	32,000	100
Black bullhead.....	-----	42	0	3	0
Green sunfish.....	2.0-8.2	0	--	2,304	100
Bluegill.....	5.5-9.2	169	100	20	100
Largemouth bass.....	6.0-15.0	56	100	14	64
Black crappie.....	7.2-8.5	1	100	14	36
Yellow perch.....	5.0-9.0	90	100	30	100

fathead minnows, carp, and green sunfish. There were partial kills of northern pike, largemouth bass, and black crappies ranging from 33 to 64 percent. Black bullheads survived.

The kill of fish was more rapid and complete in the deeper water of Pond 2, indicating a better release of the toxicant from the sand formulation. Most of the carp and other species were dead within 24 hours. We were unable to determine what influence the pollution by manure may have had on the activity of the toxicant.

There was no evidence of harm to aquatic insects in the ponds, and daphnia remained abundant, especially in Pond 2, after the treatment.

The principal highlights of the trial at Valentine were that antimycin is effective in alkaline water, in cattle-polluted water, and may eradicate carp and green sunfish at a concentration which permits survival of adult northern pike and largemouth bass.

SARATOGA, WYOMING

The Bureau's Division of Fish Hatcheries requested that we attempt to eradicate fish with antimycin in Lake Creek Lake at the Saratoga NFH. The 1.79-acre lake is spring-fed and serves as the water supply for the hatchery. The average depth is 3.25 feet, the maximum depth is 6 feet, and the water is clear and colorless. Thus, the lake afforded an excellent opportunity to further test the non-repellency of antimycin to fish and the performance of the sand formulation in

bottom springs. The hatchery's objective was to rid the lake of fish which might serve as vectors for diseases (table 11). Previous attempts with rotenone and chlorine had failed because the fish were repelled and fled into the springs to avoid contact.

The springs cause a complete exchange of water in the basin of the lake every 27 hours. Because of this, adequate exposure of fish to the toxicant was a critical consideration. Thus, we arranged to maintain at least 10 p.p.b. of antimycin in the cold, relatively hard water for a minimum of 8 hours. This dose was achieved by making an initial application of 10 p.p.b. of Fintrol-5, followed by light applications at 6 and 8 hours later to compensate for spring inflows. Uniform distributions of the sand formulation over the surface were obtained by means of a seed spreader, and the sand grains sank readily into the large, bottom springs.

The water remained clear and colorless during and after the distribution of toxicant. The fish were easy to observe, and they exhibited no alarm or tendency to escape from the treated water. The first evidence of toxic effects was apparent 2 hours after the initial application. The dense schools of large, northern creek chubs began to break up. Otherwise normal in appearance, the chubs

TABLE 11.--Effects of antimycin on fish at Saratoga NFH

Species	Size (inches)	Number present	Percent killed at 10 p.p.b.
Brook trout, small	3.2-8.2	117	100
Brook trout, large	11.2-20.1	35	100
Fathead minnow.....	2.3-3.0	several hundred	100
Creek chub.....	3.4-9.2	1,152	100
Iowa darter.....	2.5	10	100

seemed to lose their orientation with respect to schooling. None sought relief in springs. Most of them were dead within 24 hours, and all were dead within 48 hours.

Brook trout in the lake ranged from one-third ounce to 6.6 pounds, and we watched them carefully throughout the operation. They began to die after 3 hours of exposure, and all were dead within 24 hours. None had moved into the springs or tried to escape via the outlet. The responses of the numerous fathead minnows and the small numbers of Iowa darters were similar to those of the creek chubs and brook trout.

The antimycin on sand produced a complete kill of fish in Lake Creek Lake. The toxicant was flushed from the basin by spring flows within 40 hours, and it was detoxified effectively in the 3-c.f.s. outlet stream by a continual application of 1 p.p.m. of potassium permanganate for 40 hours.

WEST SALEM, WISCONSIN

Park Pond is a 4.78-acre, impounded oxbow of the La Crosse River in Veterans Memorial Park, and it is managed for trout on a put-and-take basis. It is fed by bottom springs and an artesian well with cold, very hard water. Occasionally, however, the pond is inundated by the river and becomes contaminated with carp and panfish (table 12). When abundant, the invaders are considered a nuisance, and attempts are made periodically to reduce or eliminate them.

TABLE 12.--Effects of antimycin on fish in Veterans Memorial Park Pond

Species	Size (inches)	Number present	Percent killed at 10 p.p.b.
Rainbow trout.....	9.6-11.3	6	100
Brown trout.....	7.8-13.0	54	100
Central mudminnow.....	4.0 av.	3	100
Northern pike.....	8.7-12.9	44	100
Carp, small.....	3.9-10.0	5,968	100
Carp, large.....	16.0-27.0	61	100
Golden shiner.....	4.9-6.1	49	100
Fathead minnow.....	3.3 av.	thousands	100
White sucker.....	7.8	1	100
Black bullhead.....	4.1-9.0	100+	0
Channel catfish.....	7.8 av.	2	0
Green sunfish.....	3.6	1	100
Pumpkinseed.....	2.0-4.1	106	100
Bluegill.....	2.0-3.9	19	100
White crappie.....	4.6 av.	3	100

We cooperated with the Wisconsin Conservation Department to test antimycin in the Fintrol-5 formulation in Park Pond against the numerous carp, minnows, and stunted panfish. The trial also afforded an opportunity to observe any effects of the toxicant on a large flock of semi-tame mallards on the pond.

The treatment consisted of 10 p.p.b. of antimycin and the sand formulation was distributed by seed spreader from a boat. Rainbow trout, brown trout, and northern pike were the most sensitive, and many were dead within 5 hours. Large numbers of carp and tens of thousands of fathead minnows died within 12 hours. The kill of scale fish was complete within 48 hours, and only catfishes survived.

The mallards gorged themselves on dead fathead minnows and suffered no ill effects from the toxicant or the poisoned fish. The toxicant degraded in 6 days.

Nine days after the application of antimycin, Park Pond was treated with 5 p.p.m. of rotenone to check on the kill of carp and other fish. The treatment confirmed that no scale fish survived the antimycin, and only bullheads and a few channel catfish had remained.

MADISON, WISCONSIN

Lake Katrine, a 30-acre natural lake with warm, soft water, was selected for trials of antimycin against carp eggs in the spring and for total eradication of carp and fathead minnows in the fall.

Two days after the commencement of spawning by carp, a 2.7-acre bay was treated with 4 p.p.b. of antimycin in Fintrol-5. It, and an adjacent bay serving as a control, contained hundreds of thousands of fertilized carp eggs. Within 48 hours, the treated eggs had turned white and opaque, and many were covered with fungus. In contrast, the untreated eggs were alive and reaching the eyed stage. At 96 hours, most of the treated eggs had disintegrated, and extensive sampling failed to disclose any live eggs or fry. The eggs in the control bay, however, were hatching normally and fry were abundant.

During the course of the test, some eggs from the treated and untreated bays were taken to the laboratory. Only 2 percent of the treated eggs hatched, and the fry died within 24 hours. Ninety-seven percent of the untreated eggs hatched, and the fry lived for several weeks until discarded. We concluded that antimycin has potential for killing the spawn of carp in the field.

Several months later, all of Lake Katrine was treated with 7.5 p.p.b. of antimycin in Fintrol-5 to kill carp and fathead minnows. The distribution was made in an hour by seed spreaders mounted on two boats. A heavy kill of fish was apparent within 24 hours, and sampling with large seines two weeks later indicated that the carp and fatheads had been eliminated.

OXFORD, WISCONSIN

We were asked by the Wisconsin Conservation Department to try antimycin on the carp in Parker Lake, a 63-acre natural body of water with a maximum depth of 40 feet. The problem was the abundance of young-of-the-year carp which followed an incomplete reclamation with toxaphene the previous year (table 13).

The cold, hard water was treated with 10 p.p.b. of antimycin in October. Fintrol-5 was applied in the littoral zone, 0 to 10 feet deep. Fintrol-15 and Fintrol-30 were applied to areas of the lake ranging from 11 to 20 feet deep and 21 to 40 feet deep, respectively. Some Fintrol-Liquid was sprayed on very shallow areas along the shore.

The water in the lake was clear and permitted good observation of fish. Thousands of carp and some rainbow trout and bluegills

were dead within 24 hours. Although we estimated that 60 percent of the fish were dead within 48 hours, the heavy mortality continued for 2 weeks. One month after treatment, one fingerling bluegill was caught in a fyke net. Intensive netting during the following spring failed to capture any fish, and we concluded that the antimycin had eradicated carp and other species.

The good performance of the deeper water formulations, Fintrol-15 and Fintrol-30, justified their further development for management use.

Trials in Streams

VIROQUA, WISCONSIN

The Wisconsin Conservation Department wanted to rid Sidie Hollow Creek of rough fish before filling a new impoundment which is to be managed for trout. The job offered an opportunity to test antimycin against a variety of fish in the cold, hard water of a small trout stream.

We formulated the liquid toxicant at the site by dissolving technical-grade antimycin in ethanol. By means of drip stations, a concentration of 15 p.p.b. of antimycin was maintained for 5 hours at any given site on the stream.

The concentration was too high, and it produced rapid results. Some fish exhibited distress within an hour. Most species were in distress within 3 hours, and mortality of fish was nearly complete in 12 hours (table 14). The bolt of toxicant moving downstream did not repel fish, and there was no evidence of fish fleeing ahead of the bolt. Rather, fish tended to drift involuntarily downstream when they became distressed and unable to maintain their positions in the current.

A post-treatment survey with electrofishing gear demonstrated that the 11 species of fish had been exterminated. This success led to further development and testing of Fintrol-Liquid for field application.

TABLE 13.--Effects of antimycin on fish in Parker Lake

Species	Size (inches)	Abundance	Percent killed at 10 p.p.b.
Rainbow trout.....	13-18	common	100
Carp.....	2-12	very abundant	100
Pumpkinseed.....	3-7	abundant	100
Bluegill.....	2-5	common	100
Yellow perch.....	3-9	rare	100

TABLE 14.--Effects of antimycin on fish in streams

Species	Sidie Hollow Creek 15 p.p.b.-5 hrs.		Mullet River 7.5 p.p.b.-8 hrs.		Rathbone Creek 7.5 p.p.b.-8 hrs.		Westfield Creek 10 p.p.b.-10 hrs.		Waterloo Creek 10 p.p.b.-10 hrs.	
	Abundance	Percent killed	Abundance	Percent killed	Abundance	Percent killed	Abundance	Percent killed	Abundance	Percent killed
American brook lamprey..	--	--	--	--	common	100	common	50	--	--
Rainbow trout.....	scarce	100	--	--	--	--	scarce	100	--	--
Brown trout.....	scarce	100	scarce	100	--	--	--	--	--	--
Brook trout.....	--	--	--	--	common	100	--	--	--	--
Central mudminnow.....	--	--	common	100	common	0	--	--	--	--
Northern pike.....	--	--	scarce	100	--	--	--	--	scarce	100
Stoneroller.....	common	100	--	--	--	--	--	--	--	--
Carp.....	--	--	abundant	99+	--	--	abundant	100	abundant	100
Bluntnose minnow.....	--	--	--	--	--	--	abundant	100	--	--
Fathead minnow.....	--	--	--	--	--	--	common	100	--	--
Blacknose dace.....	abundant	100	--	--	--	--	abundant	100	--	--
Longnose dace.....	common	100	--	--	--	--	--	--	--	--
Creek chub.....	common	100	common	100	--	--	common	100	--	--
White sucker.....	common	100	common	100	common	99+	abundant	100	abundant	100
Northern hog sucker.....	scarce	100	--	--	--	--	--	--	--	--
Brook stickleback.....	common	100	--	--	common	99+	scarce	100	--	--
Rock bass.....	--	--	scarce	100	--	--	--	--	--	--
Green sunfish.....	--	--	scarce	100	--	--	--	--	--	--
Pumpkinseed.....	--	--	--	--	--	--	scarce	100	--	--
Bluegill.....	--	--	scarce	100	--	--	common	100	--	--
Largemouth bass.....	--	--	scarce	100	--	--	common	100	--	--
Black crappie.....	--	--	scarce	100	--	--	--	--	--	--
Fantail darter.....	--	--	--	--	--	--	common	100	--	--
Johnny darter.....	common	100	common	100	common	100	common	100	--	--
Mottled sculpin.....	scarce	100	--	--	--	--	common	100	--	--

PLYMOUTH, WISCONSIN

The sport fishery on a portion of the Mullet River had deteriorated because of an abundance of carp, suckers, green sunfish, and other non-game fish. We cooperated with the Wisconsin Conservation Department in reclaiming a 2.5-mile portion of the river, including two small impoundments, with antimycin. Prior to the treatment, one impoundment was drained to stream bed, and the other was lowered to a pool containing 3 acre-feet of water.

The test section was characterized by a slow flow of warm, very hard water. The speed of flow was measured between application stations by use of fluorescein dye. Estimates were made on the stretchout and dilution of a toxicant between stations.

A liquid formulation of antimycin in acetone was prepared at the site. We installed drip stations and tried to maintain a concentration of 7.5 p.p.b. for a minimum of 8 hours at any given point along the stream. The 3 acre-feet of impoundment were treated with Fintrol-5 coincident with the arrival of the flowing bolt of toxicant. When the toxicant reached the downstream dam, both dams were closed. The

refilling of the impoundments thus allowed dilution and degradation of the toxicant.

Creek chubs, white suckers, green sunfish, and small black crappies began to die after 2 hours of exposure. Carp were distressed within 2 to 5 hours near sites where the toxicant was introduced. Many adult carp moved into shallow water and took up to 72 hours to die. Nine tons of carp were collected from less than 2 miles of the stream.

An electrofishing survey made 5 days after the reclamation turned up seven carp, two of which appeared to be in distress from antimycin. We estimated that more than 99 percent of the carp and 100 percent of the other fish had been eliminated by the toxicant (table 14).

The relatively slow and incomplete kill of carp indicated that the concentration of toxicant was marginal. We surmise that the loss of toxicant between fortifying stations was greater than estimated. Thus, the concentration of antimycin was less than 7.5 p.p.b. in places. This error points up the necessity of accurately measuring the stretchout and dilution of a toxicant in streams and fortifying as indicated to maintain an effective concentration along the entire course of target water.

CATARACT, WISCONSIN

Rathbone Creek provided a different experimental situation than the previous streams. It is a small, soft water, trout stream flowing through an infertile sand plain. The object of the reclamation was to reduce or eliminate white suckers to enhance intensive management of the stream for brook trout.

The 6 miles of test water were characterized by sand bottom and boggy shoreline. Two ponds were included; the upper one was drained to stream bed; and the lower pond, at the downstream limit of test water, was partially drained to a pool of 12 acre-feet. The salt-resistivity technique was used to estimate the amounts of toxicant needed at several stations to maintain a concentration of 7.5 p.p.b. of antimycin for a minimum of 8 hours at any given point in the stream. The pool in the lower pond was treated with Fintrol-5 coincident with the arrival of the bolt of toxicant in the stream. The selection of concentration and duration of exposure was based on the fact that the water temperature was only 5°C.

Dead and dying fish appeared in the following order after the treatment commenced: brook trout in 2 to 3 hours, white suckers in 3 to 6 hours, brook sticklebacks and minnows in 5 to 6 hours (table 14). All rainbow trout and fathead minnows in live cages throughout the treatment area were dead within 24 hours. The results, however, were assessed by electrofishing several days later. One fingerling white sucker, two brook sticklebacks, and numerous mudminnows were collected in four sample areas totalling 0.75 miles of stream.

An incomplete distribution of antimycin in the numerous spring seeps and backwaters may have enabled the sucker and sticklebacks to survive. It was apparent, nevertheless, that more than 99 percent of each species succumbed to the toxicant. On the other hand, the mudminnows were unaffected by the treatment. A subsequent bioassay of antimycin

against mudminnows from Rathbone Creek showed that an 8-hour exposure to 15 p.p.b. was necessary to kill them. Since mudminnows were eliminated by 10 p.p.b. of antimycin in other field trials, we concluded that the Rathbone strain was relatively resistant.

WESTFIELD, WISCONSIN

The experimental reclamation of a 3-mile portion of Westfield Creek was an attempt to eradicate the abundant carp and suckers (table 14). Power dams are located at the upper and lower ends of the test portion, and the volume of stream flow ranged from 8 c.f.s. at the upper end to 22 c.f.s. at the lower end. The pool of the lower pond was drained to stream channel. Rhodamine-B dye was used to estimate the stretchout and dilution of a toxicant.

Because of the relatively high pH and total hardness of the water, we elected to apply 10 p.p.b. of antimycin in Fintrol-Liquid formulation and to maintain the concentration for at least 10 hours. Most of the toxicant was metered into the stream, but two crews in boats endeavored to spray antimycin on backwaters and pockets off the mainstream.

The target carp and suckers began to exhibit distress in about 8 hours, and many were observed swimming slowly with the current. In the meantime, large numbers of creek chubs, other minnows, bluegills, large-mouth bass, and darters were drifting helplessly downstream. We estimated that 98 percent of the fish in the stream were dead at 24 hours, and all were dead at 96 hours. Approximately 23,000 pounds of fish were collected and carp comprised 95 percent by weight.

An electrofishing survey a week after the reclamation resulted in the capture of one brook stickleback and a few small minnows. We believe that these specimens had passed through the upper power dam after the treatment. The target species, carp and suckers, were absent.

MADISON, WISCONSIN

Waterloo Creek was selected to test the effects of a brief exposure to antimycin on carp. A 1-mile portion of the small, very slow stream was blocked off by anchored seines, and observations on flow characteristics were made with rhodamine-B dye. The experimental nature of the reclamation was accented by the fact that the water contained a heavy load of effluent from a municipal, sewage-treatment plant.

A concentration of 10 p.p.b. of antimycin was maintained for 5 hours in the test portion of stream. Fish reacted rapidly, and carp and white suckers began to die within 2 hours. Within 24 hours, thousands of dead carp and suckers and a few northern pike littered the shores, and we found no survivors (table 14). No other species were present.

Although the water at 25⁰ C. was warmer than in any of the previous field trials, we believe that it was not the sole cause for the rapidly toxic action of the antimycin. The sewage effluent may have enhanced the activity of the toxicant or reduced the resistance of the fish.

The dose of antimycin at 10 p.p.b. for 5 hours was considered very effective on carp in this situation.

Effects on Non-target Animals

Every opportunity was taken to observe any effects of antimycin on invertebrate and vertebrate animals. In most situations, the fish-killing concentrations of antimycin had no significant effects on invertebrates (table 15). The first of two exceptions occurred at Cape Vincent NFH when autumn pulses of rotifers, water fleas, and copepods were greatly reduced in ponds treated with 3 and 10 p.p.b. of antimycin. Although we are inclined to believe that the toxicant contributed to the loss of zooplankton, the season's first killing frosts at the time of the experiment may have played some part in the declines.

TABLE 15.--Effects of antimycin on invertebrates

Technical name	Common name	Effect	Concentration of antimycin (p.p.b.)
Rotatoria.....	Rotifers	99 percent kill	3.12
Tubificidae....	Aquatic earthworms	no effect	3.12
Cladocera.....	Water fleas	99 percent kill	3.12
<u>Daphnia</u>		99 percent kill	10.40
<u>Ceriodaphnia</u>		no effect	3.12
<u>Bosmina</u>		99 percent kill	10.40
		no effect	3.12
Copepoda.....	Copepods	99 percent kill	3.12
Ostracoda.....	Seed shrimps	no effect	10.40
Amphipoda.....	Scuds	Partial mortality no effect	4.0 1.04
Decapoda.....	Crawfish Freshwater shrimp	no effect Partial mortality	10.0 10.0
Ephemeroptera..	Mayflies	no effect	10.4
Odonata.....	Damselflies	no effect	10.0
Hemiptera			
Corixidae....	Water boatmen	no effect	10.4
Notonectidae..	Backswimmers	no effect	10.4
Diptera			
Tendipedidae..	Midge	no effect	12.0

TABLE 16.--Effect of antimycin on vertebrates exposed to treated water or which fed on fish killed by antimycin

Technical name	Common name	Effect	Concentration of antimycin (p.p.b.)
Ambystomidae....	Mole salamanders	no effect	10.0
Ranidae.....	Frogs	no effect	10.0
Tadpoles		no effect	10.4
Adults			
Chelydridae.....	Turtles	no effect	10.0
Colubridae.....	Water snake	no effect	10.0
Ardeidae.....	Hérons	no effect	10.4
Anatinae.....	Surface feeding ducks	no effect	10.0
Aythinae.....	Diving ducks	no effect	10.0
Larinae.....	Gulls	no effect	10.4
Sterninae.....	Terns	no effect	10.0

The second exception involved the freshwater shrimp at Saratoga NFH. Most of the shrimp in the pond were exposed to 10 p.p.b. of antimycin without harm. Some, however, were exposed to temporarily higher concentrations in springs, and they perished.

Vertebrates exposed to antimycin during the field trials have included frogs, salamanders, water snakes, turtles, waterfowl, and wading birds (table 16). Many were observed eating antimycin-killed fish. We saw no evidence of mortality or harm among these animals.

DISCUSSION

Antimycin proved to be an effective toxicant for many species of fish in lakes and streams. We endeavored to estimate a minimum effective concentration for the target species in each trial, and as might be expected, we were too high with some concentrations and too low with others. The results, however, permit some generalizations. For example, less toxicant may be required in soft water than in hard water; less is required in warm water than in cold water; and less is required in water with low pH than high pH. Prime target species such as carp and suckers can be eliminated by concentrations of 3 to 10 p.p.b. of antimycin, depending on water quality and temperature. Goldfish, bowfin, and gar are more resistant and may require considerably more than 10 p.p.b. for control. Bullheads and catfish are the most resistant, and they were relatively unharmed by the concentrations used in the trials. The differences in sensitivity of various species to antimycin are leading to tests of the chemical as a selective toxicant.

The reactions of fish to antimycin are slow and unspectacular. The first stages of distress are characterized by the fish losing their orientation and swimming slowly along the shore or near the surface. They have progressively less reaction to stimuli until they can be picked up easily by hand. In streams, they drift involuntarily downstream, feebly trying to maintain their position. They soon lose their equilibrium and turn over on their sides or backs. The final stages are characterized by short alternate periods of quiescence and feeble, erratic swimming. Infrequently, a fish will thrash about on the surface for short periods of time.

The most sensitive species such as trout and yellow perch usually show symptoms of distress in a few hours whereas more resistant ones such as largemouth bass and carp may not be affected for 10 to 24 hours. Mortality of fish is usually complete within 72 to 96 hours after treatment.

Our trials to date have indicated that the effects of antimycin are irreversible. Once

fish exhibit symptoms of distress, they die, even if placed in fresh water. In some streams where the chemical moved on past the fish, they lived for many hours in fresh water before dying.

In no case did we see fish exhibit an avoidance reaction to antimycin. They do not seek out springs or untreated areas. The lack of repellency is a significant advantage in spring-fed lakes and in streams.

The formulations of antimycin on sand are effective, easy to apply, and safe to handle. They are almost dust-free when broadcast by hand or by means of mechanical seed spreaders. Fintrol-5 was registered in the United States and Canada, largely as a result of the trials reported here. The formulations for deeper water, Fintrol-15 and Fintrol-30, are to be developed further and registered.

The results in one pond at Stuttgart, Arkansas, and another at Valentine, Nebraska, demonstrated that Fintrol-5 should not be used in water less than 3 feet deep when the bottom is very soft. Under such conditions, the sand grains may sink into the mud before releasing all of the antimycin. The concentration of toxicant in the water may be less, therefore, than desired. A liquid formulation would be better in these circumstances.

The liquid formulation of antimycin performed well in shallow waters of ponds and marshes and in streams. It can be sprayed over the water or dispensed into the water by a metering pump. A surfactant in the formulation aids dispersion in the water. We are confident that Fintrol-Liquid will be registered by the U.S. Department of Agriculture in the near future.

The evaluation of a candidate fish toxicant in the field is difficult because the techniques involved in the reclamation of lakes or streams are primitive, inexact, or unproven. The volumes of lakes are more often estimated than measured. Estimates on the volume of flow and velocity in streams are frequently and seriously inaccurate. The salt-resistivity and dye-fluorometer techniques

for detecting the flow characteristics of streams need further development to make them more widely applicable and useful. The influences of water chemistry and temperature on toxicants are not well understood, and additional research is required. The means of dispensing toxicants in various formulations need improvement. Also, more attention must be given to defining the best ways for measuring pre- and post-treatment populations of fish to improve the assessment of reclamations. And, field techniques must be developed for detecting and measuring concentrations of toxicants in lakes and streams.

The deficiencies in the technology of reclamation have caused fishery managers to resort to overdoses of toxicants to obtain satisfactory results. This is a poor and hazardous solution to the problem, and it is unacceptable to authorities who regulate pesticides.

In retrospect, the outstanding lesson derived from the field trails was that lakeside or streamside bioassays of a toxicant should be made against target species immediately prior to a reclamation. Preferably, the fish and the water in the bioassays are taken from the target lake or stream. Also, the temperatures of the bioassays and the target water should be approximately the same. Moreover, several concentrations of the toxicant which bracket the proposed concentration should be tested. The results of such bioassays should indicate the concentration and formulation of toxicant which are most likely to produce the desired results in the reclamation; the presence of favorable influences of water quality or temperature; and the presence of unusually sensitive or resistant strains of target fish.

The routine use of lakeside or streamside bioassays would benefit efficiency and economy because the number of imperfect reclamations would be reduced or the overdosing of target waters would be decreased. The idea of on site bioassays is not new. The procedure was recommended, for example, in reclaiming streams by Lennon and Parker

(1959) and in anesthetizing fish in the field by Schoettger and Julin (1967).

CONCLUSIONS

1. Antimycin is effective as a fish toxicant in standing and flowing waters.
2. The effective concentration for most species, including such prime target species as carp, suckers, and green sunfish, is 10 p.p.b. or less in waters below pH 8.5. Catfishes are not vulnerable, however, to concentrations which are lethal to scale fishes.
3. Fish eggs are killed by the same concentrations used to control free-swimming fish.
4. Antimycin degrades rapidly, especially under alkaline conditions, and most waters can be restocked with fish within 2 weeks after treatment.
5. Antimycin in water can be detoxified by 1 p.p.m. or less of potassium permanganate.
6. The effects of antimycin in fish appear to be irreversible.
7. The toxicant in liquid or sand formulations does not repel fish.
8. The concentration of toxicant to be used in a reclamation should be determined by preliminary bioassays on site against target fish in target water.
9. Antimycin in fish-killing concentrations is largely specific to fish and causes no harm to most of the other aquatic animals.
10. Concentrations greater than 10 p.p.b. are required in waters of high pH and alkalinity.

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INVESTIGATIONS IN FISH CONTROL

**28. Use of Antimycin
for Selective Thinning of
Sunfish Populations in Ponds**

By Ralph M. Burress, Fishery Biologist and
Charles W. Luhning, Physical Science Technician



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USE OF ANTIMYCIN FOR SELECTIVE THINNING OF SUNFISH POPULATIONS IN PONDS

By Ralph M. Burress, Fishery Biologist
and Charles W. Luhnig, Physical Science Technician

Bureau of Sport Fisheries and Wildlife
Southeastern Fish Control Laboratory
Warm Springs, Georgia

ABSTRACT.--Selective removal of bluegills, redear sunfish, and red-breast sunfish was accomplished in six soft-water ponds in west central Georgia by applications of 0.4, 0.6, 0.8, and 1.0 parts per billion (p.p.b.) of antimycin in the Fintrol-5 formulation. Successful treatments were made during winter, spring, and late summer at water temperatures ranging from 46° to 75° F. and under a wide variety of weather conditions. The amount of toxicant applied per treatment was calculated according to total pond volume. The 0.4-p.p.b. treatment made at 75° F. removed 2.9 pounds per acre of largemouth bass less than 6 inches long and 69 pounds per acre of all sizes of sunfishes. The 0.6 p.p.b. applied at 47° F. reduced the numbers of bluegills less than 4 inches in length by about half with very little effect on largemouth bass or larger bluegills. Bass, bluegills, and redear sunfish which survived multiple exposures to antimycin in concentrations up to 1.0 p.p.b. were able to reproduce normally. The toxicant is easy to apply, and costs only \$0.64 per acre-foot at the 0.4-p.p.b. concentration.

During the past 15 years, fishery managers have made increasing use of the technique of selectively thinning overcrowded sunfish populations in small impoundments by applying rotenone in shallow areas (Swingle, Prather, and Lawrence, 1953; Hooper and Crance, 1960; Bennett, 1960; Thomaston, 1962). Although the technique is fairly effective under favorable circumstances, its usefulness is rather seriously limited by weather conditions, water temperatures, and other uncontrollable factors which strongly influence fish behavior and distribution within the impoundment. Because of these limitations, there is only a relatively short time each year when the method can be employed. The results of this study indicate that light applications of antimycin can be used to accomplish selective

thinning of sunfish populations over a much longer period of time each year and under a much wider range of weather and water conditions than previously has been possible.

The purpose of the tests was threefold: (1) To determine what segments of the fish population would be eliminated by different concentrations of antimycin, (2) to measure the effects of given concentrations of antimycin under different experimental conditions, and (3) to learn whether fish exposed to sublethal concentrations of antimycin would reproduce normally.

The tests were conducted from December 1966 to September 1967 in six privately owned ponds which contained unbalanced fish populations. The ponds are in the vicinity of Warm

Springs, Ga., and they were referred to us by the Work Unit Conservationist, Pine Mountain Soil Conservation District.

Our sincere thanks are expressed to the following whose cooperation made this study possible: Mr. P. A. Gantt, whose help in obtaining the use of ponds for experimental purposes was invaluable; Messrs. W. A. Biggers, Joe Henry, Render Hill, J. F. Reynolds, W. S. Slaughter, and R. A. Todd, owners of the ponds; Wisconsin Alumni Research Foundation, owner of antimycin; and Ayerst Laboratories, New York, producer of antimycin.

METHODS AND MATERIALS

Surface areas of most larger ponds were determined by measuring aerial photographs, and volumes were estimated from soundings. Areas and volumes of the remaining ponds were calculated from field-drawn contour maps. The ponds were treated with one or more of the following concentrations of antimycin: 0.4, 0.6, 0.8, and 1.0 parts per billion (p.p.b.). Not more than three concentrations were used in a single pond, and treatments were made at intervals of not less than 1 month. Intensive efforts to recover dead fish were continued from 4 to 22 days after each application depending upon water temperatures. Surface water temperatures were taken each day, and average values were calculated for the time during which fish were collected. Henry Pond contained a dense growth of cat-tails and supported such a heavy plankton bloom that it was lowered to about half its original depth prior to the first treatment to

enhance recovery of dead fish. All other ponds were overflowing when treated. Final evaluations of the effects of treatment were made in different ways, as explained below.

PONDS AND FISH POPULATIONS

The ponds contained soft water, were spring fed, and were relatively infertile (table 1). They ranged in size from 0.69 to 9.80 acres, were from 3 to 6 feet in average depth, and overflowed at rates from 3.5 to 106.0 gallons per minute (g.p.m.).

Henry Pond contained a dense stand of cat-tail (*Typha latifolia*), and it was lowered about 3 feet prior to the first treatment to facilitate recovery of fish. Only sparse growths of rooted aquatic vegetation were present in the other ponds.

Each pond contained largemouth bass (*Micropterus salmoides*) and overcrowded populations of one or more of the following species of sunfishes: bluegill (*Lepomis macrochirus*), redear sunfish (*Lepomis microlophus*), and white crappie (*Pomoxis annularis*). Other species encountered included redbreast sunfish (*Lepomis auritus*), orangespotted sunfish (*Lepomis humilis*), golden shiner (*Notemigonus crysoleucas*), brown bullhead (*Ictalurus nebulosus*), channel catfish (*Ictalurus punctatus*), and mosquitofish (*Gambusia affinis*), (table 2). All of the ponds except Reynolds Pond were to be drained eventually and converted to production of channel catfish, hence their fish populations were expendable.

TABLE 1.--Some physical and chemical characteristics of six ponds treated with antimycin

Characteristics	Henry Pond ¹			Todd Pond			Hill Pond		Slaughter Pond	Reynolds Pond	Biggers Pond
Dates of treatment.....	12/05/66	1/13/67	3/08/67	12/08/66	1/09/67	3/07/67	1/31/67	3/07/67	3/13/67	3/27/67	9/11/67
Surface area (acres)...	0.16	0.16	0.77	--	0.69	--	2.25	--	2.24	9.80	2.48
Average depth (feet)...	3.0	3.0	4.0	--	5.0	--	5.0	--	6.0	4.1	4.7
Volume (acre-feet).....	0.48	0.48	3.08	--	3.45	--	11.25	--	13.44	40.18	11.67
Overflow rate (G.P.M.)..	5.3	5.3	5.3	--	3.6	--	3.5	--	6.6	5.5	106.0
Secchi disk.....	10	13	18	60	60	60	36	17	18	10	12
transparency (inches)											
Surface temperature (°F.)	47	50	61	52	46	57	56	59	64	68	75
Total hardness (p.p.m.)	8.0	10.0	10.0	12.0	12.0	15.0	7.0	8.0	8.0	8.0	10.0
Total alkalinity (p.p.m.)	11.0	11.0	9.0	12.0	12.0	12.0	7.5	7.5	8.0	8.0	14.0
pH.....	7.32	6.87	6.90	7.09	7.09	6.90	6.98	6.75	6.55	6.84	7.20

¹ Pond area, depth, and volume increased as pond filled during period of treatment.

TABLE 2.--Species of fish indicated by X were present in six ponds treated with antimycin

Species	Henry Pond	Todd Pond	Hill Pond	Slaughter Pond	Reynolds Pond	Biggers Pond
Largemouth bass.....	X	X	X	X	X	X
Bluegill.....	X	X	X	X	X	X
Redear sunfish.....	X	--	X	X	X	X
White crappie.....	--	--	--	--	--	X
Redbreast sunfish.....	--	--	--	X	--	X
Orangespotted sunfish..	--	--	--	--	--	X
Golden shiner.....	--	--	--	--	--	X
Brown bullhead.....	--	--	--	--	X	X
Channel catfish.....	--	X	--	--	--	X
Mosquitofish.....	--	--	--	--	X	--

APPLICATION OF TOXICANT

In each case, antimycin in the Fintrol-5 formulation was used. The toxicant, which was supplied by Ayerst Laboratories, was a 1-percent formulation of antimycin coated on sand with Carbowax. The amounts of toxicant applied per treatment ranged from 0.8 ounces to 6.56 pounds, and were based on pond volume without regard to maximum depth or amount of overflow. A boat was used to distribute the toxicant from a beaker, a hand-operated seed spreader, or a powered spreader such as described by Lennon, Berger, and Gilderhus (1967), depending upon the size of the area to be treated. We were able to make applications of antimycin without regard to the time of day since diurnal elevations in pH were small in each pond.

TREATMENT EVALUATION

Henry and Todd ponds were drained as much as possible soon after the last application of antimycin, and the remaining fish were recovered by seining and heavier application of toxicant. The percentages of the populations recovered after each application were calculated, and comparisons of relative effectiveness of each treatment made.

Draining of Hill and Slaughter ponds was postponed until September, so that reproductive success of the treated fish could be evaluated. Upon draining, the fish were recovered, processed, and the effect of the treatment was assessed.

Reynolds Pond could not be drained. The effectiveness of its treatment was judged by the numbers and sizes of bass and sunfishes

killed and collected within 5 days. Since the pond was large, a representative sample of small fish was picked up along segments of shoreline amounting to about one-fourth of the total. All larger bass and sunfish were picked up around the entire pond margin. The pond was seined 5 months later, in late August and early September, to measure reproductive success of treated fish and to check on the general condition of the fish population.

Biggers Pond will not be drained until further tests are made, hence the results of treatment were evaluated simply by comparing the numbers and sizes of bass and sunfishes recovered.

RESULTS

Because there were such wide variations in pond characteristics, dates and conditions of treatment, and methods of evaluating results, we will discuss each pond separately.

HENRY POND

The first treatment with 0.4 p.p.b. of antimycin killed about 10 pounds per acre of bluegills and redear sunfish, 99.5 percent of which were less than 4 inches long (table 3).

Although 50 percent more antimycin was used in the second treatment, results were virtually nil because of rain which caused the pond to discharge through the overflow pipe which had been lowered to dewater the pond. In addition to diluting the toxicant considerably, the runoff water caused turbidity that hampered recovery of the few fish which were killed.

The final application of 0.8 p.p.b. of antimycin was made under better conditions, and it killed all of the sunfishes except for one redear sunfish. About three-fourths of the small largemouth bass and one-third of the larger bass also died.

TODD POND

The 0.4-p.p.b. application killed no fish other than 30.1 percent of the small bluegills (table 3). Throughout the 2-week-long recovery period many of these fish were observed in distress. Their moribund condition

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TABLE 3.--Numbers and weights in pounds per acre and percentages (in parentheses) of various size groups of fishes recovered in six ponds following treatment with antimycin and at draining¹

Pond	Date	Average temp. (°F.)	Treatment	Combined sunfish species				Largemouth bass				Collection period (days)
				Lengths 1.0-3.9 in.		Lengths 4.0 or more in.		Lengths 1.0-5.9 in.		Lengths 6.0 or more in.		
				Number	Weight	Number	Weight	Number	Weight	Number	Weight	
Henry.....	12/05/66	59°	Antimycin (0.4 p.p.b.)	659.0 (8.7)	5.1	3.0 (1.0)	0.2	0 (0.0)	--	0 (0.0)	--	7
	1/13/67	54°	Antimycin (0.6 p.p.b.)	9.0 (0.1)	0.1	0 (0.0)	--	0 (0.0)	--	0 (0.0)	--	11
	3/08/67	69°	Antimycin (0.8 p.p.b.)	6,885.0 (91.2)	61.2	303.0 (98.7)	19.9	5.0 (71.4)	0.6	6.0 (31.6)	5.5	7
	3/20/67		Drained	0 (0.0)	--	1.0 (0.3)		2.0 (28.6)		13.0 (68.4)		
Todd.....	12/08/66	48°	Antimycin (0.4 p.p.b.)	1,380.0 (30.1)	8.4	0 (0.0)	--	0 (0.0)	--	0 (0.0)	--	14
	1/09/67	47°	Antimycin (0.6 p.p.b.)	801.4 (17.5)	5.4	1.4 (2.5)	TR. ²	2.9 (5.4)	TR.	0 (0.0)	--	22
	3/07/67	62°	Antimycin (0.8 p.p.b.)	2,388.4 (52.1)	15.7	55.1 (97.5)	1.5	46.4 (86.6)	0.2	7.2 (13.1)	8.7	7
	3/20/67		Drained	14.5 (0.3)		0 (0.0)	--	4.3 (8.0)		47.8 (86.9)		
Hill.....	1/31/67	52°	Antimycin (0.8 p.p.b.)	2,827.6 (95.9)	25.5	289.3 (97.0)	12.3	13.3 (78.7)	0.4	1.3 (12.2)	0.3	13
	3/07/67	65°	Antimycin (1.0 p.p.b.)	11.6 (0.4)	TR.	0 (0.0)	--	2.7 (16.0)	0.1	7.1 (67.0)	4.8	6
	9/18/67		Drained	108.9 (3.7)		8.9 (3.0)		0.9 (5.3)		2.2 (20.8)		
Slaughter..	3/13/67	65°	Antimycin (0.6 p.p.b.)	4,333.0 (88.9)	58.8	303.1 (92.6)	21.8	20.1 (34.4)	0.4	3.1 (31.6)	4.0	9
	9/07/67		Drained	542.4 (11.1)		24.1 (7.4)		38.4 (65.6)		6.7 (68.4)		
Biggers....	9/11/67	75°	Antimycin (0.4 p.p.b.)	2,627.0 ³	50.0	⁴ 263.7	19.0	264.5	2.7	1.2	0.2	4
Reynolds...	3/27/67	69°	Antimycin (0.6 p.p.b.)	6,326.5	63.3	11.8	1.2	27.4	0.1	2.5	2.2	5

¹ Reynolds and Biggers ponds were not drained following treatment.

² TR. = trace, or less than 0.1 pounds per acre.

³ This group included fish up to 4.9 inches in length.

⁴ This group included only fish 5.0 inches or more in length.

was evidenced by blotchy discoloration and by shreds of mucus which hung from their bodies giving some of them a very ragged appearance. Large bass were observed feeding on moribund bluegills on several occasions.

The 0.6-p.p.b. treatment killed an additional 17.5 percent of the small bluegills, 2.5 percent of the sparse population of larger bluegills, and 5.4 percent of the bass less than 6 inches long. Behavior and appearance of the larger bass were normal, but some of the smaller bass which did not die became much

darker in color and their eyes became somewhat grayish and opaque in appearance.

The 0.8-p.p.b. treatment eliminated nearly all remaining bluegills, and left only 8.0 percent of the small bass. About 87 percent of the adult bass survived, and more than a third of these exhibited the same darkening of body color and change in eye color previously shown by the smaller bass.

When the pond was drained, several adults were sent to the National Fish Hatchery in

Marion, Alabama, for breeding tests. None of the scores of channel catfish in the pond were harmed by the treatments.

SLAUGHTER POND

We recovered 85 pounds of fish per acre following a 0.6-p.p.b. application of antimycin (table 3). Approximately 90 percent of the bluegills of all sizes were killed, as were about a third of all sizes of bass.

We recovered 71.4 pounds of fish per acre when the pond was drained. Survival of adult redear sunfish was greater than that of adult bluegills, as was true in all ponds in which both species occurred. Reproductive success of largemouth bass and sunfishes had been good.

HILL POND

The first treatment on January 31 with 0.8 p.p.b. of antimycin killed about 96 percent of the sunfishes of all sizes, nearly 79 percent of the bass less than 6 inches in length, and 12 percent of the larger bass. Since the water was cold and deep, the recovery of fish undoubtedly was not as complete as it was following the other tests.

The second treatment amounted to 1.0 p.p.b., and was made on March 7. It killed a few more sunfish, and an additional 16 percent of the small bass and 67 percent of the larger bass.

The few surviving fish reproduced successfully during the summer, and at draining we found a total population amounting to 26.7 pounds per acre. Large bass fingerlings comprised the largest single group by weight in the population, amounting to 11.5 pounds per acre, while fingerling sunfishes amounted to 8.0 pounds per acre.

REYNOLDS POND

We recovered 64.5 pounds of sunfishes and 2.3 pounds of largemouth bass per acre following a single application of 0.6 p.p.b. on March 27 (table 3). A few mosquitofish were killed, but none of the brown bullheads were affected by the treatment.

Reproductive success of bluegills and redear sunfishes was very limited in that only four sunfish fry were taken in six short hauls with a small-mesh seine on August 29. On September 12, four 100-foot hauls made after dark with a 75-foot, 1/2-inch-mesh seine took 131 fingerling bass, 6 bass 5 to 7 inches long, 28 redear sunfish about 5 inches long, 2 bluegills 6 inches long, and 11 brown bullheads 4 to 8 inches long, but no sunfish less than 5 inches long or adult bass were taken.

BIGGERS POND

Following a single application of 0.4 p.p.b. of antimycin to this 2.48-acre pond on September 11, we recovered the following weights of fish per acre: 2.9 pounds of largemouth bass less than 7 inches long; 69 pounds of sunfishes including bluegills, redear sunfish, redbreast sunfish, and orangespotted sunfish; 9.0 pounds per acre of fingerling crappie; and 50.5 pounds of golden shiners from 4 to 9 inches in length. The treatment had no effect on channel catfish or brown bullheads. The surface temperature was 75° F. at time of treatment and conditions for recovery of fish were good, hence the effectiveness of this treatment was considerably greater than that measured in similar trials conducted in winter. Further tests are to be conducted in this pond before it is drained, hence we have no way at present to determine what percentages of the various groups of fishes were removed by the treatment.

DISCUSSION

The results of our exploratory attempts to evaluate the use of antimycin in selective thinning of sunfishes in small impoundments were quite encouraging. Trials made in six ponds under a wide variety of weather conditions and temperatures demonstrated that low concentrations of antimycin are quite selective against populations of panfishes as compared to those of largemouth bass, especially at lower temperatures. Treatments with a concentration of 0.4 p.p.b. of antimycin in December killed up to 8.4 pounds per acre of bluegills less than 4 inches long without killing larger bluegills or bass of any size.

The same concentration applied in September killed 2.9 pounds of small bass and 69 pounds of sunfishes of all sizes per acre. Remedial stocking with fingerling bass can be done easily if it is deemed necessary.

The 0.6-p.p.b. treatments made in small ponds less than 8 feet deep in cold weather removed up to 13.8 pounds per acre of the bluegills less than 4 inches long while killing only 0.01 pounds of the small bass. Similar treatments in warmer weather killed 80.6 pounds per acre of bluegills and only 4.4 pounds of bass in Slaughter Pond, and 64.5 pounds per acre of sunfishes and 2.3 pounds of bass in Reynolds Pond.

The 0.8-p.p.b. treatment proved to be excessively high for use in soft-water ponds in which pH values at time of treatment ranged from 6.75 to 6.98. Sunfish populations were virtually eliminated in three ponds by this concentration, bass less than 6 inches long were reduced by 78.7 to 94.7 percent, and larger bass were reduced by 12.2 to 31.6 percent.

The 1.0-p.p.b. treatment at a temperature of 65° F. was entirely too severe, leaving only 6.7 percent of the sunfish and 26.1 percent of the bass population.

The costs per acre-foot for treatments reported above when based on the list price of \$48.00 for an 8.25-pound can of Fintrol-5 were as follows: 0.4 p.p.b., \$0.64; 0.6 p.p.b., \$0.97; 0.8 p.p.b., \$1.28; and 1.0 p.p.b., \$1.60. If treatments of soft-water ponds such as ours were made in warm weather, there is a distinct possibility that concentrations even lower than 0.4 p.p.b. would be adequate. A treatment in summer might be especially appropriate in ponds in which neither bass nor sunfish reproduction was adequate, and in which selective removal of a sizeable percentage of the sunfishes could be followed shortly by supplementary stocking of bass fingerlings.

Trials in which fish were held in live boxes in water below the 5-foot level showed that mortality usually occurred more slowly and

sometimes was reduced in the deeper water. Thus, the concentration of antimycin should be calculated on the basis of pond volume above the thermocline rather than on total pond volume if lower levels were stratified or uncirculated by wind or other factors. Otherwise, the concentration might be higher than intended, with a correspondingly greater reduction in the fish population. In the case of Biggers Pond, for example, the 578 grams of toxicant used to produce a 0.4-p.p.b. concentration of antimycin in the entire pond volume would have produced a 0.5-p.p.b. concentration if all of the antimycin had been confined to the area above the 5-foot depth at which the thermocline was located. Had this occurred, the concentration would have been 25 percent greater than intended, which would have made a considerable difference in results. In any case, conservative calculation of the volume of water to be treated is preferable to reduce the possibility of overdosage.

Weather and water conditions were regarded as major causes for poor results following the 0.4- and 0.6-p.p.b. treatments of Henry Pond. However, there also is a definite possibility that little benefit was derived from the antimycin which was not released before the sand-formulated material sank into the soft, muddy bottom of the partially dewatered pond. Thus, lowering a pond prior to treatment with a given amount of Fintrol-5 will not necessarily yield better results than would have been achieved if the pond had not been lowered. Furthermore, complete eradication of fish often is easier to achieve in a full pond than in one which has been drained. This is true because pond bottoms usually contain irregularities which may hold enough water to keep fingerling fish alive for several days, and it is difficult to be certain that fish in all pockets of water have been eliminated.

We found conclusive evidence that large-mouth bass, bluegills and redear sunfish were able to reproduce successfully after one or more exposures to antimycin. Adult bass which were exposed to successive treatments of 0.4, 0.6, and 0.8 p.p.b. of antimycin during the period December 8, 1966, to March 7, 1967, were able to reproduce normally in

broodponds at the National Fish Hatchery, Marion, Alabama in April 1967¹. Bass, bluegill and redear sunfish which survived exposure to concentrations of 0.8 and 1.0 p.p.b. on January 1 and March 7, 1967, spawned successfully in Hill Pond, as did the same species in Slaughter and Reynolds ponds which were treated with a concentration of 0.6 p.p.b. of antimycin. One interesting observation was made in relation to the effect of antimycin on adult fish. During the period from March 7 to March 28, 30 of 39 adult bass found dead in five ponds were gravid females. Thus, females apparently are more vulnerable than males just prior to the spawning season.

We found no difference in results obtained by the three methods used to apply Fintrol-5 during these trials. The simplest and easiest method was to dribble the material out a small hole in a container held over the bow of the boat. Dispersion of toxicant was adequate in open water of the larger ponds even when successive passes of the boat were about 30 feet apart. However, in shallow, weedy areas where circulation was restricted we applied the material at intervals of not more than 10 feet in an effort to avoid gaps in coverage.

The Fintrol-5 formulation of antimycin gives very good selective control of sunfishes in soft-water ponds at comparatively low cost. The material is easy to apply, and applications can be made over a very wide range of water temperatures and with a minimum of regard for weather conditions. Much remains to be done in working out techniques suited to waters of different qualities and in devising variations in methods for application. Possibly liquid formulations of antimycin could be introduced well below the surface to reduce populations of crappie or other species that inhabit deeper water; manipulation of water flows might be used to attract or to concentrate certain species which then could be treated; concentrations of spawning fishes or nest areas might be treated; shoreline applications of antimycin might be used to cut treatment costs even further.

SUMMARY

1. Selective removal of sunfishes was accomplished in six soft-water ponds in west central Georgia by applications of 0.4, 0.6, 0.8, and 1.0 parts per billion (p.p.b.) of antimycin in the Fintrol-5 formulation. The treatments were made during winter, spring, and late summer at water temperatures ranging from 46° to 75° F. and under a variety of weather conditions.
2. Smaller fish of each species are more susceptible than adults; redear sunfish are more resistant than bluegills; and even fingerling bass are more resistant than adult sunfishes.
3. Response to treatment occurred more slowly in colder water, and all species, especially largemouth bass, appeared to be more resistant to antimycin at low temperatures.
4. The 0.8- and 1.0-p.p.b. concentrations were too high even in winter, and there are indications that concentrations less than 0.4 p.p.b. might be adequate when water temperatures are 75° F. or more and the pH is about neutral.
5. The toxicant is easy to apply, and costs only \$0.64 per acre-foot at a concentration of 0.4 p.p.b.
6. Largemouth bass, bluegill and redear sunfish which survived multiple exposures to antimycin were able to reproduce normally.
7. Use of antimycin for selective thinning of sunfishes will enable fishery managers to utilize this valuable technique for a longer time each year than previously was possible.

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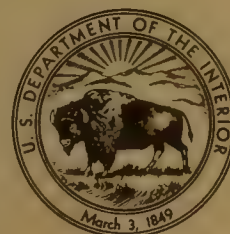
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INVESTIGATIONS IN FISH CONTROL

DIVISION OF FISHES
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29. **Efficacy of Methylpentynol as an Anesthetic
on Four Salmonids**
30. **Toxicity of Methylpentynol
to Selected Fishes**
31. **Annotated Bibliography on Methylpentynol**



**United States Department of the Interior
Fish and Wildlife Service
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29. Efficacy of Methylpentynol as an Anesthetic on Four Salmonids

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EFFICACY OF METHYLPENTYNOL AS AN ANESTHETIC ON FOUR SALMONIDS

By Robert M. Howland and Richard A. Schoettger, Fishery Biologists
Bureau of Sport Fisheries and Wildlife
Fish Control Laboratory, La Crosse, Wisconsin

ABSTRACT.--Effective concentrations of methylpentynol for anesthetizing rainbow trout, brown trout, brook trout, and lake trout were determined by a series of tests. Concentrations of 1.5 to 8 parts per thousand induced anesthesia in 4 to 57 minutes. Increase in water temperature accelerated anesthesia. Changes in pH or in water hardness had no significant effect on the rate of anesthesia. Repeated anesthesia had little effect on rate of response. The efficacy of anesthetic solutions was reduced by continuous use. Approximately 1 kilogram of rainbow trout could be effectively narcotized per milliliter of drug. Methylpentynol was compared with MS-222 as a fish anesthetic. Fifty - times as much methylpentynol was necessary to yield the equivalent effect of 100 parts per million of MS-222. Methylpentynol may be more appropriate as a sedative or soporific for salmonids than as an anesthetic.

The demand for anesthetics in stripping, marking, and transporting fish has led to experimentation with various drugs used in human medicine, such as methylpentynol. Parkhurst and Smith (1957) found that the compound worked well to quiet fish for stripping. Since then, a number of investigators have found that methylpentynol is a potent fish anesthetic and is also useful as a sedative in fish transport (Norris et al., 1960; McFarland, 1959, 1960; Carlson, 1965). However, fish entering anesthesia may struggle violently and are not completely immobilized under narcosis, according to reports cited by Bell (1964). They may also go into respiratory arrest, thereby making the maintenance of anesthesia difficult (Klontz, 1964).

Methylpentynol (3-Methyl-1-pentyn-3-ol) was originally patented in 1913 by the Bayer Company of Germany. Some common synonyms of the compound include methylparafynol, Oblivon, and Dormison (Stecher et al., 1960). During the early 1950's it was introduced as a hypnotic in human medicine (Margolin et al.,

1951; Hirsh and Orsinger, 1952; and Perlman and Johnson, 1952), but because of its side - effects and questionable efficacy (Lasagna, 1954; Marley, 1958; Kennedy and Marley, 1959; Goodman and Gilman, 1965) it had only a brief period of popularity.

Lennon (1967) discussed recent amendments to the Federal Food, Drug, and Cosmetic Act which require that chemicals used on fish be cleared and labeled for specific uses. We elected to investigate methylpentynol as one of the anesthetics because the treated fish may be eaten by humans. Information on the toxicity of methylpentynol to fish, its efficacy as a fish anesthetic, and its residues in fish tissues is necessary to obtain clearance.

We centered our investigation on the efficacy of methylpentynol, specifically the concentrations for practical use on rainbow trout, brown trout, brook trout, and lake trout. We also measured the effects of water quality, size of fish, repeated exposure, and reuse of anesthetic solutions on efficacy.

METHODS AND MATERIALS

We purchased a technical grade (Eastman grade) of methylpentynol from the Eastman Kodak Company, Rochester, N.Y. Its efficacy as a fish anesthetic was measured with trout of two size-ranges: 2 to 6 inches and 7 to 12 inches (table 1). The methods of preparing test solutions, waters of various qualities, controlling temperature, and acclimating the test fish were as described by Schoettger and Julin (1967).

The behavioral responses of trout to methylpentynol conformed reasonably well to those reported for fish exposed to MS-222 (Schoettger and Julin, 1967). Effectiveness of the drug was evaluated by placing the trout in various concentrations and recording the times for all of the test fish to exhibit the following behavioral responses: sedation; total loss of equilibrium, stage II; and loss of reflex. We also recorded the time required for the first fish to enter medullary collapse. When all fish reached this stage, they were returned to well water for recovery.

Ten fish were used to bioassay each concentration, and in most instances the experiments were replicated. The amenability of trout to handling when in the deeper stages of narcosis was tested by removing them briefly from the solutions, and simulating the stripping procedure.

TABLE 1.--Species and sources of test fish

Species	Length (inches)	Source
Rainbow trout (<i>Salmo gairdneri</i>)	2-6	Raised at Fish Control Laboratory; eggs from Rainbow Ranches, Spokane, Washington
	7-12	NFH ¹ , Manchester, Iowa
Brown trout (<i>Salmo trutta</i>)	2-6	Raised at Fish Control Laboratory; eggs from McNenny NFH, Spearfish, South Dakota
	7-12	NFH, Manchester, Iowa
Brook trout (<i>Salvelinus fontinalis</i>)	2-6	Raised at Fish Control Laboratory; eggs from SFH ² , Osceola, Wisconsin
Lake trout (<i>Salvelinus namaycush</i>)	2-6	NFH, Jordan River, Elmira, Michigan

¹ National Fish Hatchery

² State Fish Hatchery

The reusability of methylpentynol solutions was determined by anesthetizing fresh groups of five rainbow trout, 7 to 12 inches long, in 5 liters of a 5 p.p.t. (part per thousand) solution at 12°C. This concentration anesthetizes rainbow trout to loss of reflex within 2 minutes. Fresh groups of fish were exposed until the drug was no longer effective within 2 minutes. We then calculated the kilograms of trout anesthetized per milliliter of the drug.

The effect of repeated exposure on the susceptibility of rainbow trout to methylpentynol was measured by anesthetizing 10 individuals daily for 10 consecutive days at 12°C. They were narcotized to loss of reflex in a concentration of 5 p.p.t. and then placed in flowing well water for recovery. Each day, the responses of single-exposure and untreated control fish were observed in conjunction with the group undergoing repeated exposure. After 10 days, we made a gross examination for pathology in the repeatedly exposed fish, a group treated only once, and an untreated group.

RESULTS

Efficacy

Methylpentynol was 100 percent effective at concentrations of 1.5 to 8 p.p.t. for anesthetizing rainbow trout, brown trout, brook trout, and lake trout (tables 2, 3, 4, 5). These levels induce total loss of equilibrium, stage II, within 31.0 to 0.8 minutes respectively, depending on temperature, species, and size of fish. Concentrations outside this range either were ineffective or produced narcosis at rates which would be relatively impractical for most fish-handling operations.

Methylpentynol is considerably less potent as a fish anesthetic than MS-222 (McFarland, 1959). MS-222 narcotizes the species used in this study within 2 to 3 minutes at concentrations of about 100 p.p.m. at 12°C. (Schoettger and Julin, 1967). We found that at least 5,000 p.p.m. of methylpentynol were required to produce a similar effect; this concentration anesthetized the most resistant individuals in

TABLE 2.--Efficacy of methylpentynol on two sizes of rainbow trout at three temperatures

Concentration (p.p.t.)	Temperature (°C.)	Fish		Mean induction time (minutes) for			Safety index		Recovery	
		Length (inches)	Number	All fish into		First fish into	C/A ⁴	C/B ⁵	Mean time (minutes)	Percent
				Stage A ¹	Stage B ²	Stage C ³				
1.5.....	17	2-6	20	31.0	81.5	46.0	1.5	0.6	8.0	65
1.5.....	17	7-12	20	18.0	19.0	15.5	0.9	0.8	6.3	95
2.0.....	12	2-6	10	23.0	52.5	35.8	1.6	0.7	17.0	90
Do.....	17	2-6	20	7.8	16.5	12.8	1.6	0.8	10.8	90
2.0.....	12	7-12	20	18.8	22.3	23.3	1.2	1.0	9.5	80
Do.....	17	7-12	20	6.3	9.0	8.3	1.3	0.9	5.0	100
3.0.....	12	2-6	10	8.3	15.8	15.8	1.9	1.0	17.0	100
Do.....	17	2-6	20	3.0	6.0	5.5	1.8	0.9	5.3	100
3.0.....	12	7-12	20	4.8	8.0	9.0	1.9	1.1	6.5	100
Do.....	17	7-12	20	3.3	5.0	4.5	1.4	0.9	6.0	100
4.0.....	7	2-6	20	12.5	19.8	20.3	1.6	1.0	27.8	100
Do.....	12	2-6	10	4.3	8.0	8.3	1.9	1.0	14.5	100
Do.....	17	2-6	20	2.0	2.8	2.8	1.4	1.0	5.0	100
4.0.....	7	7-12	20	11.5	18.8	14.0	1.2	0.7	15.3	100
Do.....	12	7-12	20	4.0	6.5	4.5	1.1	0.7	6.8	100
Do.....	17	7-12	20	1.8	3.0	3.5	2.0	1.2	6.8	100
5.0.....	7	2-6	20	8.5	12.3	12.0	1.4	1.0	32.8	100
Do.....	12	2-6	15	2.8	5.3	6.5	2.4	1.2	15.8	100
Do.....	17	2-6	20	1.3	2.3	2.5	2.0	1.0	8.5	95
5.0.....	7	7-12	20	8.3	9.8	9.0	1.1	0.9	13.8	100
Do.....	12	7-12	20	2.0	2.5	3.0	1.5	1.2	6.5	100
Do.....	17	7-12	20	1.3	2.5	2.8	2.2	1.1	6.8	100
6.0.....	7	2-6	20	4.3	7.3	8.5	2.0	1.2	25.5	100
Do.....	12	2-6	10	1.5	3.3	3.3	2.2	1.0	16.3	100
6.0.....	7	7-12	20	6.3	8.5	7.3	1.2	0.9	15.3	100
Do.....	12	7-12	20	1.5	2.0	2.5	1.7	1.3	7.0	100
7.0.....	7	2-6	20	4.3	7.8	6.8	1.6	0.9	28.3	100
Do.....	12	2-6	10	1.0	2.0	2.8	2.8	1.4	17.8	100
7.0.....	7	7-12	20	4.5	6.5	5.5	1.2	0.8	17.3	100
Do.....	12	7-12	20	1.3	1.8	2.5	2.0	1.4	8.3	100
8.0.....	7	2-6	20	3.3	4.5	5.5	1.7	1.2	25.8	90
Do.....	12	2-6	10	0.8	1.3	2.5	3.3	2.0	17.5	100
8.0.....	7	7-12	20	4.3	5.3	5.5	1.3	1.0	18.3	100
Do.....	12	7-12	20	1.0	1.8	2.3	2.2	1.3	8.5	100

¹ Total loss of equilibrium, stage II² Total loss of reflex³ Medullary collapse⁴ Time for Stage C divided by time for Stage A⁵ Time for Stage C divided by time for Stage B

groups of 2- to 6-inch brown trout and lake trout within 3.5 minutes and the least resistant individuals among 7- to 12-inch rainbow trout and brown trout within 2 minutes. The greater sensitivity of the larger fish is even more apparent at lower concentrations (tables

2 and 3). In our opinion the size-sensitivity relation may not be valid. Large and small rainbow and brown trout were obtained from different sources (table 1); consequently interaction between size and strain may be reflected in the results.

TABLE 3.--Efficacy of methylpentynol on brown trout of two sizes at three temperatures

Concentration (p.p.t.)	Temperature (°C.)	Fish		Mean induction time (minutes) for			Safety index		Recovery	
		Length (inches)	Number	All fish into		First fish into	C/A ⁴	C/B ⁵	Mean time (minutes)	Percent
				Stage A ¹	Stage B ²	Stage C ³				
1.5.....	17	2-6	20	20.3	45.0	26.8	1.3	0.6	9.5	90
1.5.....	17	7-12	20	11.3	42.0	27.8	2.5	0.7	10.0	100
2.0.....	12	2-6	20	12.8	26.5	19.3	1.5	0.7	23.8	100
Do.....	17	2-6	20	6.8	15.5	12.8	1.9	0.8	6.5	95
2.0.....	12	7-12	20	11.8	51.0	28.3	2.4	0.6	38.0	85
Do.....	17	7-12	20	5.0	9.0	7.8	1.6	0.9	5.8	100
3.0.....	7	2-6	20	20.3	35.0	29.3	1.4	0.8	28.8	100
Do.....	12	2-6	20	10.0	13.8	11.3	1.1	0.8	17.3	90
Do.....	17	2-6	20	3.0	4.5	4.8	1.6	1.1	5.0	100
3.0.....	7	7-12	10	17.5	28.5	35.5	2.0	1.2	21.0	100
Do.....	12	7-12	20	6.0	9.5	9.3	1.5	1.0	11.0	100
Do.....	17	7-12	20	2.3	4.3	5.0	2.2	1.2	5.8	100
4.0.....	7	2-6	20	9.0	14.8	11.8	1.3	0.8	30.8	100
Do.....	12	2-6	20	5.0	7.0	5.5	1.1	0.8	12.8	100
Do.....	17	2-6	20	2.0	2.5	3.0	1.5	1.2	5.8	100
4.0.....	7	7-12	20	8.5	16.8	13.0	1.5	0.8	16.3	100
Do.....	12	7-12	20	4.0	8.5	7.0	1.8	0.8	10.3	100
Do.....	17	7-12	20	1.0	1.3	2.0	2.0	1.6	6.8	100
5.0.....	7	2-6	20	7.3	10.5	10.8	1.5	1.0	18.3	100
Do.....	12	2-6	20	3.5	4.8	5.0	1.4	1.1	12.8	100
Do.....	17	2-6	20	1.5	2.0	2.5	1.7	1.3	6.5	100
5.0.....	7	7-12	20	4.5	9.5	8.3	1.8	0.9	13.0	100
Do.....	12	7-12	20	2.0	3.8	4.5	2.3	1.2	11.8	100
Do.....	17	7-12	20	0.5	0.8	11.3	2.5	1.7	6.8	100
6.0.....	7	2-6	20	4.0	6.5	5.3	1.3	0.8	16.5	100
Do.....	12	2-6	20	1.8	2.5	2.5	1.4	1.0	8.5	100
6.0.....	7	7-12	20	3.0	7.5	5.8	1.9	0.8	11.0	100
Do.....	12	7-12	20	1.5	2.3	3.0	2.0	1.3	7.8	100
7.0.....	7	2-6	20	3.5	6.3	5.8	1.6	0.9	16.5	100
7.0.....	7	7-12	20	2.3	5.8	5.0	2.2	0.9	14.3	100
8.0.....	12	2-6	20	1.3	1.8	2.0	1.6	1.1	9.3	100
8.0.....	12	7-12	20	1.3	2.0	2.8	2.2	1.4	10.8	100

¹ Total loss of equilibrium, stage II² Total loss of reflex³ Medullary collapse⁴ Time for Stage C divided by time for Stage A⁵ Time for Stage C divided by time for Stage B

Temperature had a pronounced effect on the efficacy of methylpentynol. To illustrate, a concentration of 5 p.p.t. produced loss of equilibrium in all species within 4.5 to 8.5 minutes at 7°C., within 2 to 3.5 minutes at 12°C., and within 0.5 to 1.5 minutes at 17°C. In general, a change in temperature of 5°C. changed anesthetizing time by a factor of approximately 2 to 3.

The tractability was not consistent among the four salmonids when anesthetized to total loss of equilibrium, stage II. Lake trout and large rainbow trout were relatively immobile. The other species and sizes of fish retained considerable reflex activity, and were difficult to handle during simulated stripping procedures. Also, a state of total loss of equilibrium was inadequate for delicate surgical

TABLE 4.--Efficacy of methylpentynol on brook trout at three temperatures

Concentration (p.p.t.)	Temperature (°C.)	Fish		Mean induction time (minutes) for			Safety index		Recovery	
		Length (inches)	Number	All fish into		First fish into	C/A ⁴	C/B ⁵	Mean time (minutes)	Percent
				Stage A ¹	Stage B ²	Stage C ³				
2.0.....	12	2-6	20	25.0	54.0	42.0	1.7	0.8	26.0	80
2.0.....	17	2-6	20	12.8	31.0	28.5	2.2	0.9	12.5	90
3.0.....	7	2-6	20	22.3	34.5	34.5	1.5	1.0	26.3	100
Do.....	12	2-6	20	13.3	18.8	16.0	1.2	0.9	23.3	100
Do.....	17	2-6	20	4.8	9.5	9.8	2.0	1.0	9.0	100
4.0.....	7	2-6	20	6.5	12.3	15.5	2.4	1.3	23.0	100
Do.....	12	2-6	20	3.5	10.8	10.0	2.9	0.9	19.0	100
Do.....	17	2-6	20	2.5	4.0	4.3	1.7	1.0	10.5	100
5.0.....	7	2-6	20	4.8	13.8	13.3	2.8	1.0	23.5	100
Do.....	12	2-6	20	2.5	7.3	4.3	1.7	0.6	12.5	100
Do.....	17	2-6	20	1.3	4.5	4.0	3.2	0.9	11.3	100
6.0.....	7	2-6	20	3.3	8.0	8.3	2.5	1.0	21.0	100
Do.....	12	2-6	20	1.8	6.8	4.5	2.6	0.7	15.8	100
8.0.....	7	2-6	20	2.5	5.5	4.8	1.9	0.9	17.3	95
Do.....	12	2-6	20	1.3	3.5	2.3	1.8	0.6	12.0	100

¹ Total loss of equilibrium, stage II² Total loss of reflex³ Medullary collapse⁴ Time for Stage C divided by time for Stage A⁵ Time for Stage C divided by time for Stage B

TABLE 5.--Efficacy of methylpentynol on lake trout at three temperatures

Concentration (p.p.t.)	Temperature (°C.)	Fish		Mean induction time (minutes) for			Safety index		Recovery	
		Length (inches)	Number	All fish into		First fish into	C/A ⁴	C/B ⁵	Mean time (minutes)	Percent
				Stage A ¹	Stage B ²	Stage C ³				
1.5.....	17	2-6	20	31.5	69.8	30.8	1.0	0.4	11.0	60
2.0.....	7	2-6	10	33.5	84.5	41.0	1.2	0.5	57.3	100
Do.....	12	2-6	20	25.8	53.0	32.5	1.3	0.6	15.3	100
Do.....	17	2-6	20	9.5	23.8	10.8	1.1	0.5	7.5	50
3.0.....	7	2-6	20	12.8	25.3	19.8	1.5	0.8	26.8	100
Do.....	12	2-6	20	8.0	15.3	12.3	1.5	0.8	12.8	100
Do.....	17	2-6	20	1.8	3.3	3.0	1.7	0.9	7.5	35
4.0.....	7	2-6	20	9.0	13.5	12.3	1.4	0.9	17.5	100
Do.....	12	2-6	20	4.5	7.3	6.8	1.5	0.9	11.8	100
Do.....	17	2-6	20	0.8	0.8	1.3	1.7	1.7	4.3	80
5.0.....	7	2-6	20	5.8	5.8	8.0	1.4	1.4	14.3	100
Do.....	12	2-6	20	3.5	4.0	5.0	1.4	1.3	10.3	100
Do.....	17	2-6	20	0.5	0.5	1.0	2.0	2.0	7.0	85
6.0.....	7	2-6	20	3.0	3.0	5.3	1.8	1.8	12.3	100
Do.....	12	2-6	20	2.5	2.5	4.0	1.6	1.6	12.5	100
8.0.....	7	2-6	20	2.0	2.0	3.5	1.8	1.8	17.0	100
Do.....	12	2-6	20	1.0	1.0	1.5	1.5	1.5	12.3	100

¹ Total loss of equilibrium, stage II² Total loss of reflex³ Medullary collapse⁴ Time for Stage C divided by time for Stage A⁵ Time for Stage C divided by time for Stage B

operations on rainbow trout. (Personal communication from Dr. Joseph B. Hunn, Fishery Biologist, Fish Control Laboratory, La Crosse, Wis., October 14, 1966.)

Complete immobility of the trout can be obtained by continuing exposure to the loss-of-reflex stage. With a concentration of 5 p.p.t. at 12°C., loss of reflex usually occurs less than 3 minutes after loss of equilibrium (tables 2, 3, 4, 5). It occurs more rapidly at 17°C. and more slowly at 7°C. However, we must point out that the fish enter medullary collapse shortly after loss of reflex, particularly at higher concentrations and temperatures.

The relative risk of overexposing trout when attempting to maintain anesthesia is expressed by the safe exposure indexes shown in tables 2, 3, 4, and 5. The danger is obviously greater after the fish have entered loss of reflex, since the values are approximately 1.0 or less. In other words, in a given lot of fish, the most susceptible individuals are entering medullary collapse at the time the most resistant fish are entering loss of reflex. The exposure indexes for methylpentynol-treated trout do not appear to be correlated with concentration, temperature, species, or size of fish. By way of comparison, Schoettger and Julin (1967) reported safe exposure indexes of 2 to 3 for similar species and sizes of trout anesthetized in MS-222.

The mean times for recovery of fish from anesthesia ranged from 4 to 57 minutes (tables 2, 3, 4, 5). In general, recovery was most rapid at 17°C. and least rapid at 7°C. Most mortalities among small rainbow trout and lake trout occurred after long exposures to low concentrations at 17°C. In one instance, 35 percent of the rainbow trout died after a prolonged exposure to 1.5 p.p.t. The mortality of lake trout varied from 15 to 65 percent at this temperature, but the greatest mortalities occurred following exposures to 3 p.p.t., or less.

In all of the tests, we attempted to transfer the fish to well-oxygenated, circulating water for recovery just before their opercular movements ceased. With protracted exposures, the progression of deepening sopor in the

more sensitive fish appears to become irreversible prior to medullary collapse. This was revealed in preliminary trials at 17°C. when deaths occurred among fish which were transferred to oxygen-rich water for recovery, even though they displayed opercular rhythm at the time of removal from the anesthetic. The mortality was even greater upon transfer to unaerated, static water.

In order to avoid the rapid onset of medullary collapse associated with high concentrations, it is necessary to reduce concentration, thus sacrificing rapid anesthesia for longer handling time.

As mentioned above, there also is a risk when exposing trout to low concentrations for long periods. However, the slower rates of anesthesia at these levels permit greater control over exposure than at higher concentrations.

A good compromise between the rate of anesthesia and handling time is achieved at concentrations between 2 and 4 p.p.t. at 12°C. These relations are demonstrated in figure 1. The greatest changes in time to loss of equilibrium, stage II, loss of reflex and medullary collapse with concentration occur between 2 and 4 p.p.t. Therefore, depending on the needs,

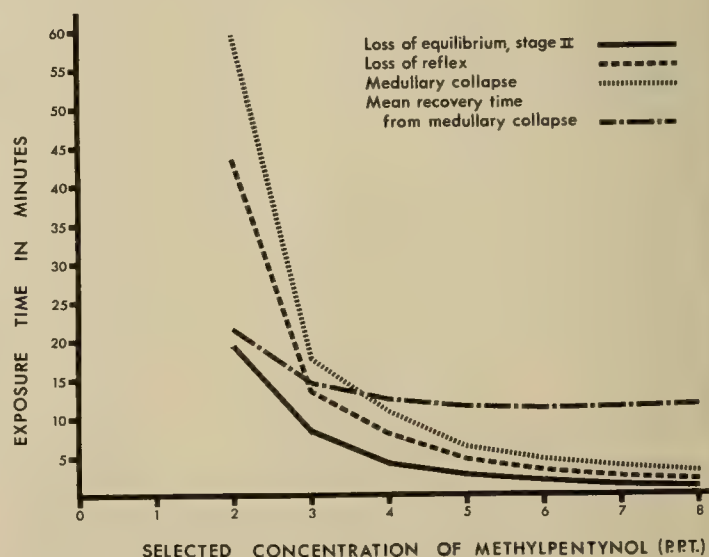


Figure 1.--Mean exposures required to induce various stages of anesthesia in trout at 12°C. for selected concentrations of methylpentynol. Curves for the response times were derived from the average of response times at each concentration for various species and sizes of trout.

the fishery worker can modify the concentration to produce the type or rate of anesthesia and handling time to meet his requirements. However, he also must consider the effects of temperature on efficacy in making an estimate of effective concentrations--a factor of about 2 to 3 occurs for each 5°C. change in temperature.

Effects of Water Quality

The efficacy of methylpentynol was neither enhanced nor decreased in bioassays on rainbow trout at 12°C. in solutions with pH's of 5, 7, and 9, and those with total hardnesses of 10, 42, and 180 p.p.m. as CaCO₃.

Repeated Use of Solutions

Five liters of a 5-p.p.t. solution of methylpentynol effectively anesthetized 260 rainbow trout, 7 to 12 inches long, at 12°C. The time required to anesthetize additional fish increased with each introduction. Thus, approximately 1 kilogram of rainbow trout can be anesthetized effectively per milliliter of drug. Meister and Ritzi (1958) reported that about 14 kilograms of brook trout and 42 kilograms of lake trout could be anesthetized per gram of MS-222.

Effects of Repeated Anesthetization

The repeated exposure of fish to methylpentynol had little influence on rate of response to the drug. From the third exposure on, the fish reacted violently upon contact with the solution, by thrashing and leaping before narcosis. Gross internal pathological examination disclosed no detrimental effects due to the anesthesia.

DISCUSSION

Our investigation establishes that methylpentynol is enigmatic as a fish anesthetic. It is capable of inducing profound stupor in four salmonids and yet it also appears incapable of producing consistently good anesthetization. The condition evoked by this drug seems

closer to inebriation than anesthesia, as evidenced by such side effects as preanesthesia and postanesthesia lunging and untoward reactions upon reanesthetization.

We stress that intractability to handling at loss of equilibrium, stage II, is pronounced in some species. Large brown trout are difficult to grasp because of tail thrashing, and small rainbow trout, brown trout, and brook trout are affected to a lesser degree. Such behavior undoubtedly impedes a practical fish-handling routine.

We corroborate the findings of Bell (1964) and Klontz (1964) regarding the struggling of fish entering narcosis, incomplete immobility, and tendency toward respiratory arrest. An explanation for these reactions is suggested in the literature. When humans are given daily doses of methylpentynol in excess of 2 grams, the drug tends to accumulate, and symptoms of intoxication appear by the third or fourth day (Goodman and Gilman, 1965). Kennedy and Marley (1959) reported abnormal brain-wave activity with toxic phenomena during a 5-day regime in humans. Also, they found a correlation between the degree of electroencephalic abnormality and the amount of physical disturbance produced by the drug in patients with toxic manifestations. A high rise of acetylcholine in rat brains, related to the degree of depression, was reported by Pepeu (1960). Margolin et al. (1951) found that methylpentynol does not possess analgesic or anesthetic properties when tested on mice and dogs, and that it lacks antispasmodic action; it was proffered solely as a soporific in human medicine.

The nature and size of the operation should determine whether this relatively expensive drug is a practical anesthetic for use in fishery management. A preferred stage of anesthesia cannot be recommended because of the aforementioned shortcomings at each level.

CONCLUSIONS

1. Methylpentynol is effective for the anesthesia of rainbow trout, brown trout, brook trout, and lake trout at concentrations of 1.5 to 8 p.p.t.

2. The rate of anesthesia increases as the concentration and/or the water temperature increases. A 5°C. change in water temperature causes a change in the rate of anesthesia by a factor of 2 to 3.
3. Concentrations from 2 to 4 p.p.t. appear better suited for handling of fish than do higher levels.
4. Water quality and pH of the anesthetic solution have no effect on the rate of anesthesia.
5. Methylpentynol does not produce the desirable or consistent characteristics of anesthesia which are acceptable for handling fish. Small lake trout and large rainbow trout were amenable to simulated stripping procedures, while small rainbow trout, small brook trout, and large and small brown trout were not.
6. Methylpentynol is less potent than MS-222 as a fish anesthetic. Fifty times as strong a solution of methylpentynol is necessary to yield the effect of a solution containing 100 p.p.m., of MS-222.
7. Methylpentynol appears to be more appropriate as a sedative or soporific for salmonids than as an anesthetic.

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INVESTIGATIONS IN FISH CONTROL

30. Toxicity of Methylpentynol to Selected Fishes

By Leif L. Marking



UNITED STATES DEPARTMENT OF THE INTERIOR
Fish and Wildlife Service
Bureau of Sport Fisheries and Wildlife
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TOXICITY OF METHYLPENTYNOL TO SELECTED FISHES

By Leif L. Marking, Chemist
Bureau of Sport Fisheries and Wildlife
Fish Control Laboratory, La Crosse, Wisconsin

Abstract.--Methylpentynol was tested in 96-hour bioassays for its toxicity to rainbow trout, brown trout, brook trout, lake trout, northern pike, channel catfish, bluegills, largemouth bass, and walleyes. The LC50's range from 660 to 1,890 parts per million at 12° C. Channel catfish are the most resistant and lake trout are the most sensitive. Two-inch rainbow trout, brown trout, and lake trout are more sensitive to methylpentynol than larger ones in 96-hour exposures. The drug is more toxic to bluegills at 17° than at 12° C. Toxicity was not influenced in different water hardnesses of 10 to 180 parts per million.

Methylpentynol (3-methyl-1-pentyn-3-ol) has been used in human medicine as a hypnotic and sedative at oral doses of 100 to 800 milligrams (Hirsh and Orsinger, 1952) and 250 to 500 milligrams (Stecher et al., 1960). Overdoses, however, may produce acute psychosis, abnormalities of the nervous system, or coma and death in extreme cases. Since methylpentynol possesses no unique advantages as a human hypnotic to outweigh its disadvantages, it is no longer recommended (Sharpless, 1965). The oral LD50 for mice, rats, and guinea pigs is 600 to 900 mg./kg. (Margolin, Perlman, and McGavack, 1951).

Methylpentynol is a recognized anesthetic for fish at concentrations of 0.5 to 0.9 ml./l. (Klontz, 1964). Others have reported applications of the drug as a hypnotic in transporting fish at concentrations of 1 to 3 ml./gal. (Bell, 1964; Carlson, 1965; Fry and Norris, 1962; and McFarland, 1959 and 1960). Bayliff and Klima (1962) reported methylpentynol toxic to some fish at 6 ml./gal. (approximately 1,580 p.p.m.) and suggested it was too slow-acting for field use. Howland and Schoettger (1969) found that 1,500 to 8,000 p.p.m. of methylpentynol produced anesthesia within 4 to 57

minutes, but suggest the compound is better suited as a sedative than an anesthetic. As an anesthetic there is danger of overexposing the fish because of the high concentrations required to achieve rapid anesthesia.

Since fish can be overdosed with methylpentynol, we should determine its toxicity to fish. Amendments to the Federal Food, Drug, and Cosmetic act also require toxicity data to clear the drug for this use (Lennon, 1967). I have chosen to determine the toxicity of methylpentynol to rainbow trout, brown trout, brook trout, lake trout, northern pike, channel catfish, bluegills, largemouth bass, and walleyes with considerations to changes in temperature, water quality, and size of fish.

METHODS AND MATERIALS

Experimental fish were obtained from State and Federal fish hatcheries (table 1). Three size groups included 1- to 3-inch, 3- to 5-inch, and 6- to 9-inch fish. Ten fish were tested at each of 10 or 11 concentrations of methylpentynol in 15-liter, static bioassays according to the methods of Lennon and Walker (1964).

TABLE 1.--Fishes used in tests of methylpentynol

Common and scientific name	Source
Rainbow trout (<i>Salmo gairdneri</i>)	Manchester NFH ¹ , Iowa
Brown trout (<i>Salmo trutta</i>)	Manchester NFH, Iowa
Brook trout (<i>Salvelinus fontinalis</i>)	Osceola SFH ² , Wis.
Lake trout (<i>Salvelinus namaycush</i>)	Jordan River NFH, Mich. and St. Croix Falls SFH, Wis.
Northern pike (<i>Esox lucius</i>)	Garrison Dam NFH, N.D. and Gavins Point NFH, S.D.
Channel catfish (<i>Ictalurus punctatus</i>)	Fairport NFH, Iowa
Bluegill (<i>Lepomis macrochirus</i>)	Lake Mills NFH, Wis.
Largemouth bass (<i>Micropterus salmoides</i>)	Genoa NFH, Wis.
Walleye (<i>Stizostedion vitreum</i>)	Garrison Dam NFH, N.D.

¹ National Fish Hatchery² State Fish Hatchery

At least 10 of the 1- to 3-inch fish served as controls. The bioassays with the 3- to 5-inch and 6- to 9-inch fish were conducted in polyethylene tanks containing 45 liters of aerated solution. Five concentrations were tested against the 9-inch fish and 10 fish served as controls.

Waters of different hardnesses and pH were prepared by adding greater or less amounts of reconstituting salts to deionized water (table 2). Temperatures of 7°, 12°, 17°, and 22° C. were maintained by placing the bioassay vessels in thermostatically controlled water baths. All temperatures are reported in Celsius.

Aliquots of Eastman-grade methylpentynol, purchased from Eastman Organic Chemicals, were pipetted into the bioassays to yield the desired concentrations.

The data on survival and mortality were recorded at 24, 48, and 96 hours and were analyzed according to the methods of Litchfield and Wilcoxon (1949) to determine LC50's, variations, slope functions, and 95-percent confidence intervals.

RESULTS

Species and Sizes of Fish

The LC50's of methylpentynol range from 660 to 1,890 p.p.m. in 96-hour bioassays for all species tested (table 3). Channel catfish, 2 and 4 inches long, are the most resistant species while small lake trout are the most sensitive.

Larger brown trout and lake trout are more resistant to the lethal effects of methylpentynol than the 2-inch ones. This is apparent with brown trout and lake trout at all observation periods, and with one lot of rainbow trout at 96 hours. The medium sized brown trout and lake trout tolerate concentrations which approximate those tolerated by the larger individuals. LC50's range from 1,060 to 1,160 p.p.m. in 96 hours for 3- to 7-inch brown trout and lake trout.

Three- and 4-inch brook trout responded uniformly to methylpentynol with 96-hour LC50's of 1,200 and 1,280 p.p.m. respectively. However, the 6-inch fish appear more sensitive in the 96-hour bioassay. This may be explained by the fact that they contracted furunculosis just prior to the bioassays. Unfortunately, the disease was not diagnosed until after the tests.

Methylpentynol acted slowly at the concentrations tested, and little or no mortality occurred within 3 hours, even at concentrations

TABLE 2.--Water qualities obtained with different amounts of reconstituting salts in deionized water

Classification of water	Salt added in mg./l.				pH range	Concentration as p.p.m. CaCO ₃	
	NaHCO ₃	CaSO ₄	MgSO ₄	KCl		Total hardness	Total alkalinity
Soft.....	12	7.5	7.5	0.5	6.4-6.8	10-13	10-13
Standard ¹	48	30.0	30.0	2.0	7.2-7.6	40-48	30-35
Medium.....	192	120.0	120.0	8.0	7.6-8.0	160-180	110-120

¹ Standard reconstituted water used in routine bioassay.

TABLE 3.--Toxicity of methylpentynol to nine species of fish at 12° C.

Species	Average weight (grams)	Approximate length (inches)	LC50 (p.p.m.) and 95-percent confidence interval at		
			24 hours	48 hours	96 hours
Rainbow trout.....	1.9	2	1,220 1,151-1,294	1,150 1,095-1,208	870 753-1,005
Do.....	1.6	2	1,340 1,276-1,407	1,280 1,219-1,344	1,250 1,214-1,288
Do.....	23.0	6	1,300 1,204-1,404	1,290 1,217-1,367	1,260 1,223-1,298
Brown trout.....	2.6	2	820 745-902	750 714-786	680 636-728
Do.....	14.3	4	1,090 1,048-1,134	1,085 1,033-1,139	1,060 1,010-1,113
Do.....	27.0	6	1,130 1,100-1,158	1,100 1,028-1,177	1,100 1,038-1,166
Brook trout.....	12.5	3	1,300 1,250-1,352	1,270 1,221-1,321	1,200 1,132-1,272
Do.....	20.0	4	1,375 1,317-1,430	1,300 1,250-1,352	1,280 1,231-1,331
Do.....	37.5	6	1,210 1,142-1,283	1,175 1,108-1,246	1,100 1,028-1,177
Lake trout.....	2.0	2	900 849-954	860 789-937	660 584-746
Do.....	5.6	3	1,280 1,143-1,434	1,230 1,108-1,365	1,160 1,084-1,241
Do.....	35.0	7	1,220 1,140-1,305	1,200 1,132-1,272	1,160 1,094-1,230
Northern pike.....	1.8	2	1,050 1,012-1,077	1,000 943-1,060	< 900 ---
Channel catfish.....	1.9	2	1,770 1,702-1,841	1,770 1,702-1,841	1,700 1,604-1,802
Do.....	5.2	4	1,890 1,817-1,966	1,890 1,817-1,966	1,890 1,817-1,966
Bluegill.....	1.3	2	1,390 1,337-1,446	1,340 1,301-1,380	1,340 1,301-1,380
Do.....	2.8	3	1,370 1,317-1,425	1,350 1,320-1,382	1,260 1,189-1,336
Largemouth bass.....	0.5	1	1,250 1,220-1,282	1,250 1,202-1,282	1,170 1,114-1,228
Do.....	5.2	4	1,270 1,233-1,308	1,185 1,129-1,244	1,100 1,028-1,177
Do.....	63.0	7	1,400 1,321-1,484	1,300 1,250-1,352	1,250 1,190-1,312
Walleye.....	0.7	2	1,225 1,156-1,298	1,160 1,048-1,288	1,140 1,027-1,265

substantially higher than those required to kill fish at 24 hours. Little additional mortality occurred among any species after 24 hours, except the small rainbow trout, brown trout, and lake trout. In fact, the LC50's are identical, or nearly identical at 24, 48, and 96 hours for some species.

Northern pike appear the most sensitive among the warmwater species. The young pike require a large supply of food and become predacious in the bioassay since no food is available. Also they become weak when not fed for 96 hours and a complete statistical evaluation could not be calculated at that time.

TABLE 4.--Toxicity of methylpentynol to rainbow trout and bluegills at different temperatures

Species	Temperature (°C.)	LC50 (p.p.m.) and 95-percent confidence interval at		
		24 hours	48 hours	96 hours
Rainbow trout.....	7	1,450 1,343-1,566	1,330 1,255-1,410	1,330 1,255-1,410
Do.....	12	1,340 1,276-1,407	1,280 1,219-1,344	1,250 1,214-1,288
Do.....	17	--	--	930 845-1,023
Bluegills.....	12	1,390 1,337-1,446	1,340 1,301-1,380	1,340 1,301-1,380
Do.....	17	1,120 1,040-1,198	1,070 1,009-1,334	1,030 928-1,143

Effects of Temperature

Methylpentynol is more toxic to 1.6-inch rainbow trout and 1.3-inch bluegills at the higher temperatures in 24- to 96-hour bioassays (table 4). The 96-hour LC50 for rainbow trout at 7° C. is 1,330 p.p.m. whereas at 17° C. the value drops to 930 p.p.m. Although bluegills are less sensitive, the toxicity of the drug increases about 20 percent with an increase in temperatures from 12° to 17° C. The LC50's for bluegills are 1,340 and 1,030 p.p.m. at 12° and 17° C. respectively at 96 hours. Little additional mortality of either species occurred after 24 hours and LC50's are fairly consistent throughout the bioassay.

Effects of Water Quality

Methylpentynol appears equally toxic to 1.9-inch rainbow trout in water containing about 11, 44, and 170 p.p.m. of total hardness at 24 and 48 hours and appears only slightly more toxic in the harder water at 96 hours (table 5). The difference appears insignificant

in soft and standard water hardness at all observation periods. The LC50's ranged from 780 to 920 in the three water hardnesses at 96 hours.

DISCUSSION

Rainbow trout and bluegills are more sensitive to the toxic effects of methylpentynol at higher temperatures. This corresponds with efficacy trials of Howland and Schoettger (1969) in which higher temperatures stimulate the metabolic activity and the drug is assimilated much faster.

Anesthesia is induced by 1,500 to 8,000 p.p.m. of methylpentynol (Howland and Schoettger, 1969). These concentrations exceed LC50's for 24- to 96-hour bioassays, but the exposure time is only 4 to 57 minutes for effective anesthesia.

Parkhurst and Smith (1957) exposed rainbow trout to 2,400 p.p.m. of methylpentynol. Undoubtedly this concentration would become toxic to the trout in longer exposures.

CONCLUSIONS

The 96-hour LC50's of methylpentynol to fish range from 660 to 1,890 p.p.m. Higher concentrations are necessary to produce effective anesthesia, but exposures are also much shorter.

Larger brown trout and lake trout are more resistant to the toxic effects of methylpentynol than smaller ones.

TABLE 5.--Toxicity of methylpentynol to rainbow trout in selected water qualities¹ at 12° C.

Water hardness ¹	LC50(p.p.m.) and 95-percent confidence interval at		
	24 hours	48 hours	96 hours
Soft.....	1,260 1,202-1,320	1,200 1,135-1,268	920 821-1,018
Standard.	1,230 1,157-1,308	1,190 1,127-1,256	980 905-1,060
Medium...	1,210 1,163-1,258	1,200 1,138-1,265	780 603-1,009

¹ Water quality described in table 2.

The toxic effects of methylpentynol are manifest in 24 hours and little additional mortality occurs after this time.

Channel catfish are the most resistant and lake trout the most sensitive to methylpentynol.

Methylpentynol is more toxic to rainbow trout and bluegills at higher temperatures.

Changes in water hardnesses of approximately 12, 44, and 170 p.p.m. influence the toxicity of methylpentynol very little.

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1962. The transporting of live fish. In Georg Borgstrom, editor Fish as Food, vol. 2, chapter 17, p. 595-608. Academic Press, N.Y.
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1960. The use of anesthetics for the handling and the transport of fishes. California Fish and Game, vol. 46, no. 4, p. 407-431.
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INVESTIGATIONS IN FISH CONTROL

31. Annotated Bibliography on Methylpentynol

By Gerald E. Svendsen



UNITED STATES DEPARTMENT OF THE INTERIOR
Fish and Wildlife Service
Bureau of Sport Fisheries and Wildlife
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ANNOTATED BIBLIOGRAPHY ON METHYLPENTYNOL

By Gerald E. Svendsen, Biologist
Bureau of Sport Fisheries and Wildlife
Fish Control Laboratory, La Crosse, Wisconsin

Abstract.--An annotated bibliography containing 26 selected references on the biochemistry, physiology, and methods of analysis of methylpentynol.

Experiments with methylpentynol as an anesthetic for four salmonids to describe its toxicity and efficacy began in 1964 at the Fish Control Laboratory, La Crosse, Wis. The U.S. Food and Drug Administration requires these data for clearance and labeling of drugs. During the study, a number of selected references on fishery uses of the drug, and on its biochemistry, physiology, and methods of analysis were annotated.

BIBLIOGRAPHY

- Bayliff, William H., and Edward F. Klima.
1962. Live-box experiments with anchovetas, *Cetengraulis mysticetus*, in the Gulf of Panama. *Inter-American Tropical Tuna Commission Bulletin*, vol. 6, No. 8, p. 333-436.
Four anesthetics--quinaldine, MS-222, methylpentynol, and tertiary amyl alcohol--were used to facilitate tagging operations. Quinaldine caused some mortalities and proved to be chemically unstable. Methylpentynol and tertiary amyl alcohol also killed some fish and acted too slowly for field use. The methylpentynol was used at a concentration of 6 milliliters per gallon. MS-222 is the most satisfactory of the chemicals tested, although there were some mortalities at the higher concentrations.
- Bell, Gordon R.
1964. A guide to the properties, characteristics, and uses of some general anaesthetics for fish. *Fisheries Research Board of Canada, Bulletin 148*. 4 p.
- The structural formulas, manufacturers, dosages, toxicities, and solubilities are presented for 11 chemicals used as fish anesthetics: carbon dioxide, chloral hydrate, chloretone, ether, methylpentynol, MS-222, phenoxyethanol, quinaldine, sodium amytol, tribromomethanol, and tertiary amyl alcohol. Methylpentynol is recommended at a dosage of 1 to 2 milliliters per liter in transportation. Bell does not recommend it as an anesthetic for surgery because immobilized fish twitch when prodded or cut. Methylpentynol immobilizes fish slowly with occasional violent struggling, but recovery is rapid in fresh water.
- Carlson, Frank T.
1965. Susquehanna River Shad study. *Pennsylvania Angler*, October, p. 1-7.
Shad were transported in a solution containing 1 milliliter of methylpentynol per gallon of water. They were anesthetized in a 15-gallon tub of river water containing 3 milliliters of methylpentynol per gallon.
- Fry, F. E. J., and K. S. Norris.
1962. The transporting of live fish. In *Georg Borgstrom, Editor Fish as Food*, Vol. 2, Chap. 17, p. 595-608. Academic Press, N.Y.
The authors suggest methylpentynol for use in transporting live fish.
- Hirsh, Harold L., and William H. Orsinger.
1952. Methyloparafynol- a new hypnotic. Preliminary report on its therapeutic efficacy and toxicity. *American Practitioner*, vol. 3, no. 1, p. 23-26.

Patients received a 100 to 800 milligram dose of methylpentynol as a sedative with no after effects on blood pressure, pulse, respiration rate, blood and urine composition, electrocardiogram, or liver and kidney function. The authors considered methylpentynol a safe, nontoxic, efficient, rapid, and long lasting hypnotic drug in humans.

Howland, Robert M., and Richard A. Schoettger.

1969. Investigations in Fish Control:

29. The efficacy of methylpentynol as an anesthetic on four salmonids. U.S. Bureau of Sport Fisheries and Wildlife.

Methylpentynol was tested as an anesthetic against rainbow trout, brown trout, brook trout, and lake trout. Concentrations ranging from 1.5 to 8.0 parts per thousand produced anesthesia within 4 to 57 minutes respectively. They studied the effects of water temperature, water quality, and pH on the rate of anesthetization and found that only temperature had a measurable effect. The authors concluded that methylpentynol is better suited as a sedative than as an anesthetic for salmonids, because fish under anesthesia are not completely immobilized.

Job, C. von.

1959. Die Beziehungen zwischen der stärke der narkotischen wirkung und der thermodynamischen konzentration bei estern des methylpentynols. *Arzneimittel-Forschung*, vol. 9, no. 1, p. 14-22.

The author investigated the effects of methylpentynol and some of its esters on the rat and on the isolated frog nerve. Action potentials of the isolated frog nerve are retarded and interrupted under the influence of methylpentynol. Methylpentynol blocks conduction of stimuli at a concentration of 5 grams per liter.

Kennedy, Walter A., and Edward Marley.

1959. The electroencephalographic effects of methylpentynol. *Electroencephalography and Clinical Neurophysiology*, vol. 2, no. 1, p. 59-64.

The authors discuss several aspects of the drug in clinical work, and cite cases of overdosage and side effects from methylpentynol. They found a correlation between the degree

of electroencephalographic abnormalities and the amount of physical disturbance produced in patients given 0.5 gram of methylpentynol orally for 5 days.

Klontz, George W.

1964. Anesthesia of fishes. In *Proceedings of the Symposium on Experimental Animal Anesthesiology*. Brooks Air Force Base, December 14-16. 13 p.

Techniques for anesthetizing fish and a brief description are given for 15 agents: carbon dioxide, electricity, diethyl ether, secobarbital sodium, amobarbital sodium, urethane, chloral hydrate, tertiaryamyl alcohol, tribromoethanol, chlorobutanol, 2-phenoxyethanol, 4-styrylpyridine, methylpentynol, quinaldine, and MS-222. Concentrations of 0.5 to 0.9 milliliter per liter of methylpentynol are suggested to induce anesthesia within 2 to 3 minutes. Maintenance of anesthesia is considered fair, and the recovery of fish in fresh water occurs in 5 to 20 minutes. The author also rated the maintenance of deep anesthesia as fair because fish seem to go into respiratory arrest. He considered the odor of methylpentynol quite disagreeable.

Lasagna, Louis.

1954. A comparison of hypnotic agents.

The Journal of Pharmacology and Experimental Therapeutics, vol. III, p. 9-20.

The author used chloral hydrate, pentobarbital sodium, methylpentynol, and a placebo to determine which doses are most useful for inducing prolonged sleep in man. A dosage of 0.5 to 1.0 gram of methylpentynol induced sleep that was undistinguishable from that of the placebo.

Leal, Aluisio Marques, and Maria Helena Diniz.

1956. Assay of methylpentynol in galenic preparations. *Revista Portuguesa de Farmacia*, vol. 6, p. 14-17.

Two titrimetric methods for the assay of methylpentynol in body fluids are described.

Margolin, S., P. Perlman, F. Villani, and T. H. McGavack.

1951. A new class of hypnotics: unsaturated carbinols. *Science*, vol. 114, p. 384-385.

Methylpentynol was studied for use as a clinical hypnotic. The LC_{50} for mice, rats, and guinea pigs is 600 to 900 mg./kg. (milligrams per kilogram). The animals died in coma. Two hundred to 300 mg./kg. had no effect on mice, rats, and dogs. One half to 4.6 percent of a 200-mg./kg. dose fed to dogs was excreted in the urine within 24 hours. Ten minutes after an intravenous administration of 200 mg./kg. to dogs, 20 percent of the dose is found in the blood, but none is present after 2 hours. Twenty percent of an 800-mg./kg. dose is found in the muscle and liver tissues taken from rats which are still under hypnosis. No residues were found in these tissues after the effects of anesthesia wore off. Methylpentynol was not metabolized by whole blood of dogs or rats but it was metabolized by slices of kidney, liver, or brain.

Marking, Leif L.

1969. Investigations in Fish Control: 30.

The toxicity of methylpentynol to selected fishes. U.S. Bureau of Sport Fisheries and Wildlife.

Toxicity of methylpentynol to rainbow trout, brown trout, brook trout, lake trout, northern pike, channel catfish, bluegills, largemouth bass, and walleyes of various sizes ranged from the 96-hour LC_{50} value of 660 p.p.m. (parts per million), for the more sensitive lake trout to 1,890 p.p.m. for channel catfish at 12° C. Larger rainbow, brook, and lake trout were considerably more resistant than smaller ones. Rainbow trout and bluegills are more sensitive to methylpentynol in warmer temperatures. Total hardnesses of 10.0 to 170.0 p.p.m. produced similar results in the static bioassays. Methylpentynol is much less toxic than other anesthetics tested in the 24-, 48-, and 96-hour static bioassays at selected temperatures and water qualities.

Marley, E.

1959. Pharmacology of methylpentynol and methylpentynol carbamate. *British Journal of Pharmacology*, vol. 14, p. 284-306.

Methylpentynol and methylpentynol carbamate are depressants of monosynaptic and polysynaptic reflexes in cats, frogs, rabbits, and guinea pigs. Small doses exerted weak ganglionic and neuromuscular blocking actions,

increased aortic blood flow, diminished systolic amplitude, increased coronary flow, and stimulated respiration. Large doses depressed respiration.

Marley, E., and W. D. M. Paton.

1959. The effect of methylpentynol and methylpentynol carbamate on the perfused superior cervical ganglion of the cat. *British Journal of Pharmacology*, vol. 14, p. 303-312.

The output of acetylcholine in the perfused cervical ganglion is depressed by dosages of 1 to 5 milligrams of the drugs.

Marley, E., and J. R. Vane.

1958. The distribution of methylpentynol and methylpentynol carbamate in tissues and body fluids of cats. *British Journal of Pharmacology*, vol. 13, p. 364-371.

The authors present a modified titrimetric method for estimating methylpentynol in amounts as small as 0.1 milligram. They found no difference between plasma concentrations and whole blood concentrations of methylpentynol 10 minutes after injection. They concluded that the drug has free access to all parts of the body, and general anesthesia does not inhibit its metabolism and excretion. It enters cells easily, where it tends to accumulate.

Marley, Edward.

1958. Susceptibility to methylpentynol and methylpentynol carbamate. *British Medical Journal, Medical Memoranda*, August 23, p. 493.

He compared the toxicities of methylpentynol and methylpentynol carbamate in man. Methylpentynol at 0.5 gram per day for 5 days causes many toxic effects in man.

McFarland, William N.

1959. A study of the effects of anesthetics on the behavior and physiology of fishes. *Publications of the Institute of Marine Science, University of Texas*, vol. 6, p. 23-55.

The anesthetic effects of 21 chemicals on *Fundulus parvipinnis*, *Gambusia affinis*, *Paralabrax alathratus*, and *Girella nigricans* were investigated. The effects on behavioral patterns are observed in four major stages: sedation, loss of equilibrium, loss of reflex,

and medullary collapse. These stages are compared to the sequence of anesthesia described for higher vertebrates. Narcotic potencies are correlated with molecular weight of the drugs. Methylpentynol is rated highly potent.

McFarland, William N.

1960. The use of anesthetics for the handling and the transport of fishes. *California Fish and Game*, vol. 46, no. 4, p. 407-431.

The author suggested that MS-222, tertiary amyl alcohol and methylpentynol are beneficial for inducing deep anesthesia because the drugs act quickly and the fish recover rapidly. Recovery is complete, provided the respiratory movements have not ceased for more than a few minutes. A concentration of 1.5 to 2.0 milliliters per gallon of methylpentynol is considered desirable for transporting marine and freshwater fishes. Methylpentynol lowers metabolic rates and therefore increases load capacity. He suggests that fishes should be pretreated in the anesthetic to reduce metabolic rates prior to loading and transportation.

Nicholls, J. G., and J. P. Quilliam.

1956. The mechanism of action of paraldehyde and methylpentynol on neuromuscular transmission in the frog. *British Journal of Pharmacology*, vol. 11, p. 151-155.

Paraldehyde and methylpentynol block neuromuscular transmission by decreasing the secretion of acetyl cholinesterase at the synapse in the frog.

Norris, Kenneth S., Frank Brocato, Frank Calandrino and William N. McFarland.

1960. A survey of fish transportation methods and equipment. *California Fish and Game*, vol. 46, no. 1, p. 5-33.

Methylpentynol is suggested as a useful anesthetic in fish transportation.

Parkhurst, Z. E., and M. A. Smith.

1957. Various drugs as aids in spawning rainbow trout. *The Progressive Fish-Culturist*, vol. 19, no. 1, p. 39.

A methylpentynol concentration of 2,400 p.p.m. caused sluggishness and relaxation in

rainbow trout. The trout are ready for spawning in 3.5 minutes at a water temperature of 43° F. Somewhat longer exposures are not harmful. Trout remained in good condition 75 days after spawning. There was an 84.1 percent hatch from those fish spawned with drugs, and an 84.0 percent hatch from controls.

Pepeu, Giancarlo, and Nicholas J. Giarmann.

1960. Effect of methylpentynol on acetylcholine in the rats brain. *Nature*, vol. 186, p. 638.

The authors found that methylpentynol did not interfere with the synthesis of acetylcholine in the rat brain, nor did it inhibit cholinesterase activity. Male rats were given intraperitoneal injections of methylpentynol in a dosage varying from 200 to 500 milligrams/kilogram.

Perlman, Preston L., and Carol Johnson.

1952. The metabolism of Dormison (3-methyl-pentyne-ol-3, methyl-parafynol) and methods for the estimation of Dormison in biological materials. *Journal of the American Pharmaceutical Association*, vol. 41, no. 1, p. 13-16.

The authors described a tritrimetric method for estimating methylpentynol in biological fluids and tissue. They studied the metabolism of methylpentynol in dogs. Methylpentynol is metabolized by destruction of the ethinyl group, which metabolizes quite rapidly. This is revealed by the rapid decline in the blood level of methylpentynol and the slow level of elimination of the chemical in the urine, and *in vitro* by the disappearance of the ethinyl group by metabolizing rat tissues. They found no evidence of storage or accumulation of the drug in tissues.

Perlman, Preston L., David Sutter, and Carol B. Johnson.

1953. Further studies on the metabolic disposition of Dormison (3-methyl-1-pentyn-3-ol) in dogs and man. *Journal of the American Pharmaceutical Association*, vol. 42, no. 2, p. 750-753.

Dogs eliminated 17-27 percent of the administered methylpentynol conjugated with glucuronic acid; 1 percent unchanged in the urine, and none by way of the lungs or feces.

In man, up to 10 percent is eliminated unchanged, and 17-27 percent as conjugates of glucuronic acid. The peak levels for elimination of the drug are reached within 48 hours.

Quilliam, J. P.

1959. Paraldehyde and methylpentynol and ganglionic transmission. *British Journal of Pharmacology*, vol. 14, p. 277-283.

Paraldehyde and methylpentynol block transmission of the impulse at preganglionic nerve terminals in cats. The author suggests that this is caused by a decrease in the secretion of acetylcholine from the preganglionic nerve terminals.

Schafferzich, S., and Beverly J. Brown.

1952. Anticonvulsant activity and toxicity of methylparafynol (Dormison) and some other alcohols. *Science*, vol. 116, p. 663.

The authors used phenobarbitol, 2-methyl-2-propanol, 2-methyl-2,4-pentamediol, 3-pentanol, 2-methyl-2-butanol, and methylpentynol as an anticonvulsant in treatment of epilepsy. Rats were not effected by 0.17 percent methylpentynol in their drinking water

for 4 months. The LD₅₀ of methylpentynol to rats is 300 to 900 milligrams per kilogram. They concluded that methylpentynol is the least safe of those chemicals studied.

Sheldon, J. M.

1965. Plastic bag transport of salmon and steelhead by air and car. *The Progressive Fish-Culturist*, vol. 27, no. 2, p. 86.

The author used methylpentynol, 0.67 milliliter per gallon of water, as a sedative to transport salmon and steelhead in plastic bags. Three milliliters of 10 percent Dow-Corning Antifoam emulsion was added to prevent excessive foaming.

Smith, J. N., and R. T. Williams.

1954. The metabolism of aliphatic alcohols. The glucuronic acid conjugation of chlorinated and some unsaturated alcohols. *Biochemistry Journal*, vol. 56, p. 618-621.

They studied the conjugation of glucuronic acid with a number of chlorinated and other aliphatic alcohols in the rabbit. About 50 percent of the methylpentynol is conjugated with glucuronic acid and excreted in the urine.

(Reports 18 through 21 are in one cover.)

18. Toxicity of 22 Therapeutic Compounds to Six Fishes, by Wayne A. Willford. 1967. 10 p.
19. Toxicity of Bayer 73 to Fish, by Leif L. Marking and James W. Hogan. 1967. 13 p.
20. Toxicity of Dimethyl Sulfoxide (DMSO) to Fish, by Wayne A. Willford. 1967. 8 p.
21. Labor-Saving Devices for Bioassay Laboratories, by Robert J. Hesselberg and Ralph M. Burress. 1967. 8 p.

(Reports 22 through 24 are in one cover.)

22. Efficacy of Quinaldine as an Anesthetic for Seven Species of Fish, by Richard A. Schoettger and Arnold M. Julin. 1969. 10 p.
23. Toxicity of Quinaldine to Selected Fishes, by Leif L. Marking. 1969. 10 p.
24. Quinaldine as an Anesthetic for Brook Trout, Lake Trout, and Atlantic Salmon, by David O. Locke. 1969. 5 p.

(Reports 25 through 28 are in one cover.)

25. Field Trials of Antimycin as a Selective Toxicant in Channel Catfish Ponds, by Ralph M. Burress and Charles W. Luhning. 1969. 12 p.
26. Laboratory Studies on Antimycin A as a Fish Toxicant, by Bernard L. Berger, Robert E. Lennon, and James W. Hogan. 1969. 19 p.
27. Field Trials of Antimycin A as a Fish Toxicant, by Philip A. Gilderhus and Robert E. Lennon. 1969. 21 p.
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As the Nation's principal conservation agency, the Department of the Interior has basic responsibilities for water, fish, wildlife, mineral, land, park, and recreational resources. Indian and Territorial affairs are other major concerns of this department of natural resources.

The Department works to assure the wisest choice in managing all our resources so that each shall make its full contribution to a better United States now and in the future.

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